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Molecular Cytogenetics of TEL/AML1 Fusion and other Abnormalities Involving TEL and AML1 Genes in Pediatric Acute Lymphoblastic Leukemia

SHERINE I. SALEM, M.D.* and HANY ABD EL-RAHMAN, M.D.**

The Department of Clinical Pathology and Pediatric Oncology**, National Cance Institute, Cairo University.*

ABSTRACT

Background: The TEL/AML1 fusion is the most common genetic abnormality found in childhood acute lymphoblastic leukemias (ALL). This cryptic translocation is not detected by conventional cytogenetic techniques but it can be readily detected using fluorescence in situ hybridization (FISH). We carried out cytogenetic and FISH studies on 50 children with ALL in order to measure the frequency of this translocation in the Egyptian population in comparison to other genetic subgroups, the incidence of TEL and/or AML1 gene alterations and their correlation with clinical evolution and prognosis.

Design and Methods: Bone marrow samples were obtained from 50 pediatric ALL patients who Karyotyping and FISH using probes for TEL/AML1, BCR/ABL and MLL were performed. The signal pattern of AML1 and TEL genes were analyzed using fluorescent in situ hybridization with a dual color DNA probe specific for the AML1 and TEL genes. Patients were treated according to the National Cancer Institute protocols and followed up for a period of 18 to 42 months (median 19 months).

Results: In the current study, successful karyotyping was obtained in 43/50 (86%); when adding FISH 46/50 (92%) cases showed informative results. Using FISH, TEL/AML1 fusion was detected in 7/50 cases (14%) while cytogenetics could not reveal the translocation (12;21). Normal karyotype was found in 13/50 (26%) when using conventional cytogenetics (CC) only, while when combining both FISH and CC two cases revealed TEL/AML1 gene fusion. Other chromosomal abnormalities that were frequently encountered in TEL/AML1 positive cases were either, deletion of chromosome 12p arm in 2 cases (4%), and polysomies of chromosome 21 in 2 cases (4%). These abnormalities correspond to lack of TEL signal and extra AML1 signals respectively as detected by FISH.

Also CC failed in 7 cases (14%), while when FISH was performed MLL gene translocation was detected in one case and TEL/AML1 gene fusion in 2 cases. Hyperdiploidy was found in 11 cases (22%), t(1;19) in 4 cases (8%), BCR/ABL, MLL gene translocation, t(8;14) in 3 cases (6%) each, hypodiploidy in 2 cases (4%) and AML1 gene amplification in one case (2%).

Best overall survival correlated with TEL/AML1 positive cases and high hyperdiploid cases with mean overall survival (OS) being 28.3 and 26.3 months respectively. Worst overall survival was associated with all other chromosomal abnormalities having mean OS of 14.6 months (p=0.0134). Cases with normal karyotype had a mean OS of 22.2 months, while cases with abnormalities of TEL or AML1 genes other than TEL/AML1 fusion had a mean OS of 21.8 months.

Conclusion: TEL/AML1 fusion is found in 14% in our series and had a favorable outcome. Other TEL and AML1 genes abnormalities are detected in 14/50 cases (28%) and associated with intermediate OS.

Key Words: *ALL – TEL/AML1 – AML1 amplification – TEL deletion – Fluorescence in situ hybridization.*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood, accounting for approximately 20% of all childhood malignancies in the National Cancer Institute. It is well established that the identification of cytogenetic abnormalities is very useful for the risk stratification of childhood ALL. For instance, t(9;22), 11q23/MLL gene abnormalities, and hypodiploidy are known to confer a poor prognosis and t(12;21), hyperdiploidy are associated with a favorable outcome **[1]**.

The translocation (12;21) (p13;q22) which results in TEL/AML1 (ETV6/RUNX1) fusion, is a common genetic abnormality in acute lymphoblastic leukemia (ALL) **[2-5]**. It is detected in about 20 to 25% of cases **[6,7]**. Although it is difficult to identify by conventional cytogenetic techniques, TEL/AML1 fusion can be readily detected using fluorescence in situ hybridization (FISH) **[2,3]**. This translocation is

associated with an early onset of the disease, a Pre B-lineage immunophenotype and a favorable prognosis **[1,8]**. However, questions about the relationship between t(12;21) and favorable prognosis have arisen because several patients with TEL/AML1 fusion showed poorer clinical outcome if other gene rearrangements coexist **[9,10]**.

Additional cytogenetic abnormalities, associated with the t(12;21), are frequently found. The most common structural and numerical aberrations are 12p rearrangements and trisomy 21, respectively **[11-13]**. A double fusion signal, detected by FISH, is another common finding in patients with t(12;21) **[3,6]**. Deletions involving the untranslocated TEL allele, detected by cytogenetic and/or FISH techniques, are also often found in patients with TEL/AML1 fusion (3,15). Although deletions on the short arm of chromosome 12 are recurrent alterations found in a wide range of hematologic neoplasias, the leukemogenic role of the genes located there, TEL and p27, is unknown **[16]**.

Other studies reported intrachromosomal duplication of chromosome 21 and amplification of the RUNX1 (AML1) gene (iAMP21) as a recurrent chromosomal abnormality with an incidence of 1.5% in childhood B-lineage acute lymphoblastic leukemia (ALL) **[17]**. These patients have a median age of 9 years, a low presenting white blood cell count and a poor prognosis **[18]**. Thus, on the current U.K ALL treatment protocol, ALL 2003, these children are classified as high-risk and receive more intensive treatment **[19]**.

The RUNX1, formerly known as AML1 gene is also involved in many chromosomal aberrations associated with hematologic disorders. More than 40 different patterns of translocations or rearrangements involving the 21q22 region have been described **[20]** and amplification of the 21q22 region in pediatric patients with ALL has also been detected by comparative genomic hybridization (CGH) **[21]**. Thus, the AML1 gene could have an important role in the 21q22 amplification in childhood ALL **[21-22]**.

Twin studies on ALL patients **[23,24]** and retrospective analysis using neonatal blood spots (Guthrie cards) **[25-27]** have provided evidence that TEL/AML1 often arises prenatally, possibly as a first or initiating event. Consequently, "preleukemic" clone with TEL/AML1 can persist postnatally for extended periods, up to 14 years **[23]** and that at least one other postnatal genetic event is required for overt leukemia. Deletion of the nontranslocated TEL allele is the most common secondary abnormality found in newly diagnosed TEL/AML1-positive cases of ALL **[6,14,28]**. Studies on both singletons **[6,28]** and twins **[24]** with ALL indicate that such TEL deletions are subclonal or secondary to TEL/AML1 and almost certainly postnatal. Candidate preleukemic clones in normal cord blood with TEL/AML1 fusions retain the normal TEL allele **[29]**. Whether other genetic changes in addition to TEL deletion, e.g., kinase mutations **[30]**, are necessary for the clinical development of ALL remains to be established.

In the present study, we performed karyotyping and FISH with probes for BCR/ABL, MLL and TEL/AML1 rearrangements, for 50 childhood ALL cases. The aim of this study was to estimate the frequency of TEL/AML1 rearrangement among other genetic subgroups involving the above genes and to study the impact of any additional abnormalities and correlate with the clinical outcome.

MATERIAL AND METHODS

Patients:

Bone marrow samples were obtained from 50 (29 males and 21 females) pediatric newly diagnosed with B-lineage acute lymphoblastic leukemia between 2004 and 2006. The leukemia immunophenotype was determined by standard flowcytometric analysis using a panel of monoclonal antibodies. All patients were classified according to the WHO classification criteria.

Treatment protocol:

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, 6000 u/m^2 alternating days, 3 times a week, for 9 doses, Triple intrathaecal: Methotrexate, Cytarabine and Hydrocortisone, given on days 1, 43. Etopside (VP16) and cyt-

arabin (Ara-C): 300mg/m^2 IV, each were given on days 22, 25, 29. Bone marrow examination for re-evaluation 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 300 mg/m^2 IV gave weekly starting from W1 till W61. Mercaptopurine (6MP) 75 mg/m^2 PO, for 7 days + Cytoxan 300 mg/m^2 IV 4 weekly from week 65 till week 117. 6MP IM 8 weekly starting from week 2 till week 118. MTX: 40 mg/m^2, IM + Ara-C: 300 mg/m^2 IV, 8 weekly start from week 3 till week 115. VCR IV, 1.5mg/m^2 + L-asparagenase:IM, 10000 u/m^2 once + Prednisolone: PO, 40mg/m^2 for 7 days given 4 weekly basis starting from week 4 till week 36, the coming weeks only VCR + Prednisolone were given on weeks 40, till week 120 on 4 weekly basis. HD-MTX: IV, 500mg/m^2 over 1 hour followed by 1500 mg/m^2 over 23 hours + 6MP: 75 mg/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: 75 mg/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 performed by bone marrow analysis and CSF examination, 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.

Patients were followed up for a period of 18 to 42 months (median 19 months).

Conventional cytogenetics:

Bone marrow or blood samples preserved on sodium heparin were cultured for each subject as previously described **[31]**. Chromosomes were incubated at 37ºC in culture media for 24 to 48 hours. Colcemid was added to arrest cells at mitosis, followed by addition of a hypotonic solution (KCl). Cells were then fixed with Carnoy Fixative, dropped onto clean slides, trypsin banded and karyotyped using an image analysis system (Vysis Quips XL Genetic work station) according to the international system of hemocytogenetic nomenclature **[32]**. Fifty patients with B-lineage ALL who were treated between 2004 and 2006 according to the NCI protocol were included in the study.

FISH:

FISH was performed using a selected panel of commercial probes for LSI TEL/AML1 ES Dual Color Translocation Probe (Vysis Inc., Downers Grove, IL, U.S.A), LSI BCR/ABL Dual Fusion Translocation Probe (Vysis) and MLL Dual Color Break Apart Rearrangement Probe (Vysis). FISH was performed according to the manufacturer's instructions. The image was analyzed using Vysis Quips XL Genetic work station. Metaphases and at least 200 interphase nuclei were analyzed for each case. Nuclei with ambiguous signals were excluded from analysis.

Statistical analysis:

Statistical Package for social sciences (SPSS) version 9 was used. Quantitative variables were summarized using mean, median, minimum and maximum values. Qualitative data were summarized using frequencies and percentage. Chi or Fisher's exact tests were used whenever appropriate to test the association between the different qualitative variables. Differences were considered significant at a p value of ≤0.05 and highly significant at a p value of ≤0.01.

RESULTS

The clinical and biological characteristics of the 50 patients are summarized in Table (1). The median age at diagnosis was 6 years (range 2 months -18 years) including 4 infants (<1 year). The immunophenotyping was precursor B ALL in all cases. Patients were subjected to conventional cytogenetics and FISH using probes to detect TEL/AML1, BCR/ABL and MLL rearrangements. Successful kayotyping was obtained in 43/50 (86%), while when using FISH 46/50 (92%) of cases showed informative results. Normal karyotyping was obtained in 13/50 (26%) of cases by conventional cytoge-

netics (CC), while in 11/50 (22%) when combining both CC and FISH. Chromosomal abnormalities were detected in 30/50 (60%) by CC while when combining both CC and FISH, 35/50 (70%) abnormalities were detected. High hyperdiploidy was detected in 11/50 (22%) of cases, TEL/AML1 in 7/50 (14%) and t(1;19) in 4/50 (8%) and t(8;14), BCR/ABL, MLL gene rearrangement in 3/50 (6%) each. BCR/ABL was detected as a part of complex karyotype (case 1), as a sole abnormality (case 4) and as double Philadelphia chromosome in a hyperdiploid karyotype (case 6) (Figs. 3,4). The MLL gene rearrangement was in the form of t(4;11) in one case (case 2) (Fig. 5), t(10;11) as a part of complex karyotype (case 28), while the third case had no mitotic figures and only FISH could detect the MLL translocation (case 29). Hypodiploidy (less than 45 chromosomes) was detected in 2 cases. One of the hyperdiploid cases had BCR/ABL gene fusion as well. Two cases having normal karyotype, showed TEL/AML1 when FISH was performed. FISH also revealed MLL gene translocation in one case and TEL/AML1 gene fusion in 2 cases with failed karyotype (Tables 2, 3).

In the TEL/AML1+ patients, 2 cases showed normal karyotype, in 2 cases karyotyping was not successful and 3 cases presented with abnormal karyotype (Fig. 1). Secondary chromosomal abnormalities were detected in 4/7 (57%). These abnormalities were +del 21 (extra fusion signal) in one cases (Fig. 2), +21 (extra AML1 signal) and del12p (deletion of untranslocated allele of TEL) in two case.

In TEL/AML1- patients, 14 cases showed either loss or gain of TEL and/or AML1 genes. Three cases showed TEL gene anomalies; case number 2 had TEL deletion together with MLL gene translocation, case number 38 showed monosomy 12 and hence one copy of TEL gene with a hypodiploid karyotype and case number 23 had trisomy 12 and thus 3 copies of TEL gene. Gain of the AML1 gene was detected in 10 cases; in cases 21 and 23 tetrasomy of chromosome 21 leading to 4 copies of AML1 gene. In case 32 there was amplification of AML1 gene and in cases (3, 5, 6, 11, 15, 17, 20, 25) trisomy of chromosome 21 and hence 3 copies of AML1 gene were detected as a part of a hyperdiploid clone with or without structural abnormalities. AML1 amplification was detected in a 6 year old boy with TLC $5.9x10^9$/L who did not achieve complete remission and died one month after start of therapy.

Ten cases showed numerical chromosomal abnormalities; one of them had TEL/AML1 gene fusion as revealed by FISH. Eleven cases showed structural chromosomal abnormalities, while 9 cases had both numerical and structural abnormalities. FISH revealed clonal chromosomal abnormalities in five more cases with either normal or failed karyotype.

The current study also revealed two cases with less common chromosomal abnormalities; del 6q, -20 (case 10) (Fig. 6) and del 9p (case 48).

Clinical correlation:

Age, sex and total leucocytic count (TLC) are summarized in Tables (4, 5 and 6). Response to induction chemotherapy and overall survival are presented in Table (7) and (Fig. 7). Complete remission was achieved in 41/50 (82%) cases with a mean OS of 20.6 months. The other 9 cases included 2 (4%) cases with normal karyotype, 2 (4%) cases with BCR/ABL, 2 (4%) cases with t(1;19), one (2%) case with t(8;14), one (2%) case with amplification of AML1 gene and one case with failed karyotype.

All 7 patients with TEL/AML1 fusion achieved complete clinical remission. They had a mean age of 4.3 years, range 0.2-18 years, TLC mean was $39.6X10^9$/L, range 2-$189X10^9$/L. Mean overall survival 28.3 months, (p= 0.0757), range 18-35 months and were all alive at the end of the study (Table 2).

The 10 cases with high hyperdiploid karyotype {not including t(9;22)} achieved CR with a mean OS of 26.3 months, range 17-42 months. They had a mean age of 10 years, range 2.5-18 years, TLC mean was $30.46X10^9$/L, range 1.4-$103X10^9$/L and were all alive in CR at the end of the study except two patients who died in CR.

Normal karyotype was encountered in 11/50 cases, 9 of them achieved complete remission, while one case entered into myelosuppression and died in CR (case 40) and another one (case 41) died shortly after diagnosis. Their mean OS was 22.2 months, range 1-30 months, they had

a mean age of 6.7 years, range 0.2-18 years, TLC mean was 64.0X10^9/L, range 3-289X 10^9/L.

Eighteen patients with t(9;22), MLL gene rearrangement, t(8;14), hypodiploidy, AML1 gene amplification were grouped together due to limited number of cases and their mean overall survival was 14.6 months (Fig. 8), significantly lower than the rest of the patients (p= 0.0134), range 1-44 months. They had a mean age of 9.7 years, range 0.3-18 years. TLC mean was 61.2X10^9/L, range 1.8-400X10^9/L. Cytogenetic results could not be obtained in 4 case (cases 14, 16, 19 and 47). Apart from TEL/AML1 positivity, 14 cases showed either AML1 or TEL aberrations as revealed by FISH. All cases achieved complete clinical remission, except for 2 cases (cases 6 and 32).

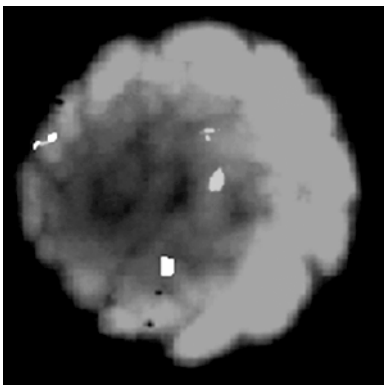

Fig. (1): FISH showing translocation TEL/AML1 using LSI TEL/AML1 extra signal dual color probe (vysis).

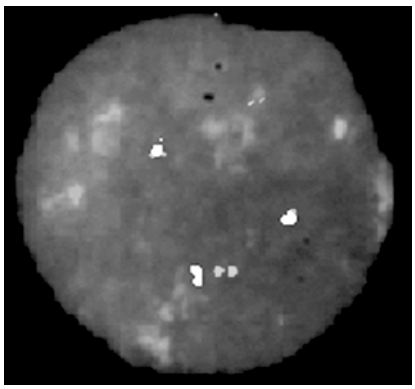

Fig. (2): FISH using LSI TEL/AML1 extra signal dual color probe (vysis) showing translocation TEL/ AML1 with an extra fusion signal denoting an extra derivative 21.

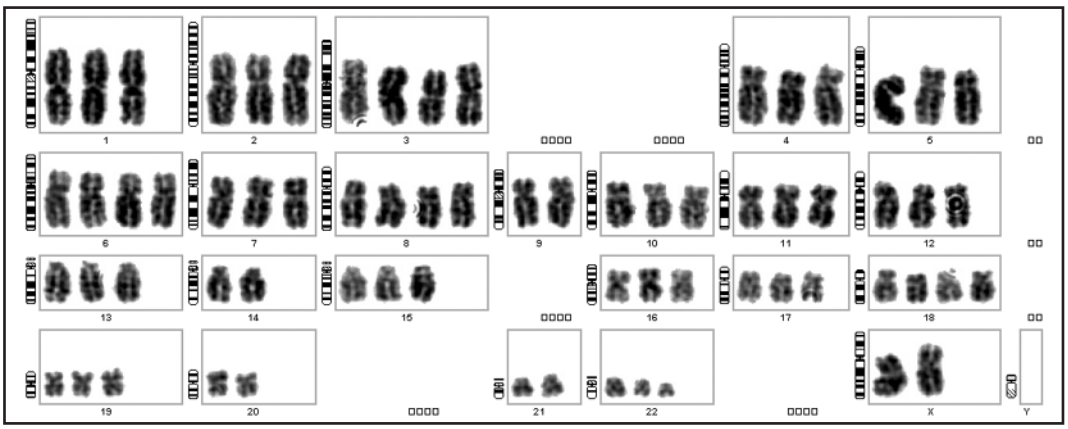

Fig. (3): Karyotype of case No. 6 showing High Hyperdiploidy and double Philadelphia chromosome.

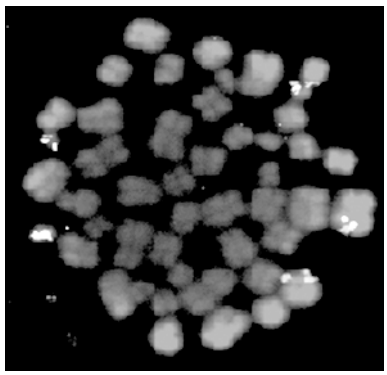

Fig. (4): FISH of the same case using BCR/ABL dual fusion probe showing high double Philadelphia chromosome.

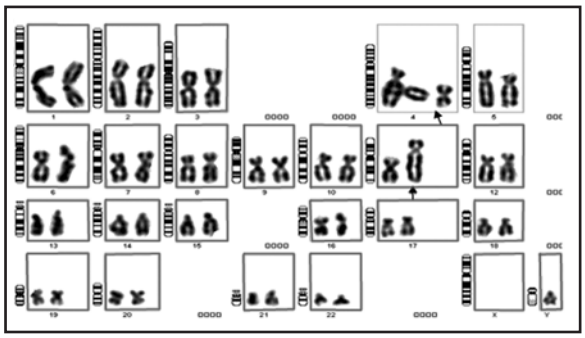

Fig. (5): Karyotype of case No. 28 showing t(4;11) (q21;q23).

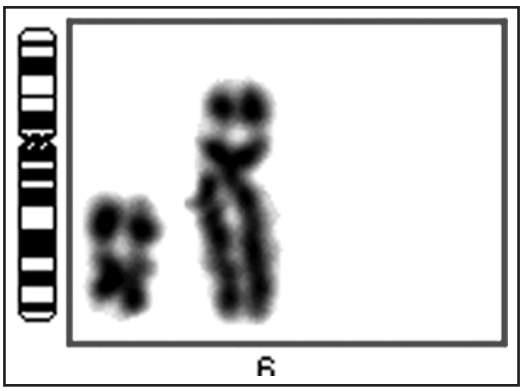

Fig. (6): Partial karyotype of case No. 10 showing deletion of the long arm of chromosome 6.

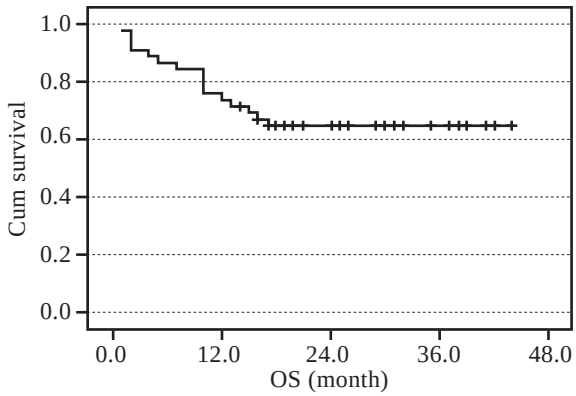

Fig. (7): Overall survival of all patients.

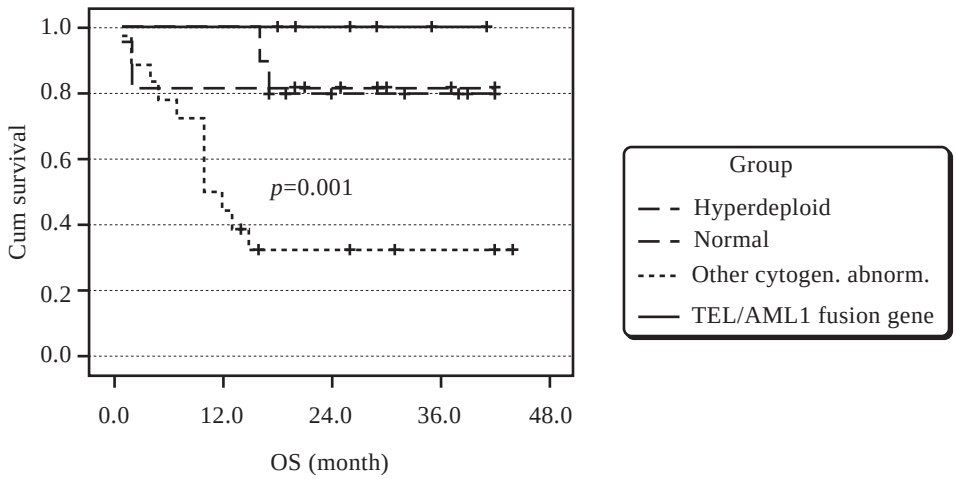

Fig. (8): Overall survival of different cytogenetics group.

Table (1): Clinical and biological characteristics of 50 B lineage ALL patients with different cytogenetic findings.

	Total	Normal karyotype	High hyperdiploidy	TEL/AML+	Others*
No. cases	50	11/50 (22%)	10/50 (20%)	7/50 (14%)	18/50 (36%)
Gender:					
Male	29 (58%)	7 (63.5%)	4 (40%)	5 (71.5%)	12 (66.6%)
Female	21 (42%)	4 (36.5%)	6 (60%)	2 (28.5%)	6 (33.3%)
Median	25.5	4	9	3.8	11
Age	0.2-18	0.2-18	2.5-18	2-10	0.3-18
Range		p=0.4719	p=0.4561	p=0.1117	p=0.4682
Age:					
<1	4 (8%)	1 (9%)			2 (11.1)
1-9.9 yrs	25 (50%)	7 (63.6%)	5 (50%)	6 (85.7%)	6 (33.3%)
10-18 yrs	21 (42%)	3 (27.3%)	5 (50%)	1 (1.3%)	10 (55.6%)
TLC ($x10^9$/L):	35 (70%)	6 (54.5%)	8 (80%)	6 (85.7%)	12 (66.6%)
<50	15 (30%)	5 (45.5%)	2 (20%)	1 (1.3%)	6 (33.3%)
≥50		0.6976	0.3745	0.6680	0.7012

*Cases with BCR/ABL, MLL gene rearrangement, hypodiploidy, t(1;19), t(8;14) and other less common cytogenetic abnormalities.

Table (2): Secondary chromosomal abnormalities in 7 TEL/AML1 positive precursor B-ALL patients.

No. cases	Age/ sex	TLC	Karyotype	TEL deletion	Trisomy 21	Induction response	Overall survival (month)	Status
9	3/M	189	46, XY			CR	26	Alive
13	2/M	6.2	49, XY, +8, +21, +mar		+	CR	18	Alive
31	4/F	11	46, XX			CR	29	Alive
37	3.8/M	20	47, XY, +21		+	CR	20	Alive
43	5/M	14	46, XY, del12p	+		CR	35	Alive
45	2/F	17	Not done			CR	41	Alive
46	10/M	20	Not done	+		CR	29	Alive

Table (3): Chromosomal abnormalities in 43 TEL/AML1 negative B-ALL patients.

No.	Age	TLC	Karyotype	BCR/ABL	MLL*	RR	OS	Status
1	18/M	1.8	46, XY, (1;10) (q12;q21), t(9;22) (q34;q11), -14, del17 (p11), +mar	+		CR	10	D
2	0.8/M	7.1	46, XY, t(10;11) (q22;q23), del (12) (p11), -17, -19, -20, +22, +mar, +mar		+	CR	12	D
3	2.5/F	19	55~56, XX, +X, +4, +6, +7, +8, +10, +11, +13, +21, +mar			CR	42	A
4	18/M	12.8	46, XY, t(9;22) (q34;q11)	+		R	4	D
5	7/F	24	62, XX, +X, +4, +6, +8, +10, +10, +11, +13, +14, +16, +17, +18, +20, +21, +22, +mar			CR	16	D
6	16/F	14.4	69, XXX, +3, +5, +8, t(9;22) (q34;q11)x2, +10, +18	+		R	7	D
7	0.3/F	17.8	46, XX, t(1;19) (q23;p13)			R	10	D
8	3/M		46, XY, der(19) t(1;19) (q23;p13)			CR	14	A
10	12/F	400	46, XX del6q, -20, +mar			CR	26	A
11	11/F	103	67, XXX,…			CR	24	A
12	1/F	21	46, XX, t(1;19) (q21;p13)			R	5	D
14	5.2/M	19.3	Failed			CR	14	D
15	16/F	96	55, XX, +3, +6, +8, +9, +11, +15, +20, +21, +22			CR	39	A
16	2.5/M	179	Failed			R	4	D
17	17/F	10.1	52, XX, +8, +11, +17 +18, +19, +21			CR	17	A
18	0.2/M	13	46, XY			CR	25	A
19	16/F	14	Not done			CR	16	A
20	3.5/M	6.2	56, XY, +6, +8, +10, +13, 14, +17, +18, +20, +21, +22			CR	19	A
21	4/M	7.1	65, XXY, +2, +3, +4, +6, +8, +9, +10, +11, +12, +13, +15, +16, +18, +19, +20, +21, +21, +22			CR	38	A
22	3/M	71	46, XY			CR	20	A
23	17/M	24.2	89, XXYY……			CR	19	A
24	4/M	14	46, XY			CR	17	A
25	18/M	1.4	52, XY, +4, +6, +10, +14, +18, +21			CR	17	D
26	4/F	13.6	57, XXX, +4, +8, +12, +14, +15, +16,+17, +20, +21, +mar			CR	32	A
27	15/M	102	45, XY, add1q, t(8;14), -11, -17, +mar			R	13	D
28	10/M	104	46, XY, t(4;11) (q21;q23)		+	CR	42	A
29	0.3/M	2.3	Not done		+	CR	10	D
30	3.5/M	44	46, XY, t(8;14) (q24;q32)			CR	44	A
32	6/M	5.9	47, XY, +mar			R	1	D
33	16/M	168	46, XY, t(8;14) (q24;q32)			CR	15	D
34	2/M	289	46, XY			CR	42	A
35	7/M	19	46, XY			CR	21	A
36	11/M	30	46, XY			CR	29	A
38	18/F	2.9	42, XX, -2, -12, -14, -19			CR	16	A
39	18/F	76	46, XX			R	2	D
40	18/M	32.9	44, XY, -8, -13			CR	2	D
41	18/M	66	46, XY			R	1	D
42	2/F	12	46, XX, t(1;19) (q23;p13)			CR	10	D
44	6/F	24	46, XX			CR	37	A
47	14/F	35	Not done			CR	15	D
48	16/M	97	46, XY, del9p			CR	31	A
49	3/F	41	46, XX			CR	20	A
50	2/F	61	46, XX			CR	30	A

*All three cases showed MLL gene translocation.

Table (4): Age distribution of patients with positive karyotype at presentation.

Age	Case summaries		
	N	Mean	Std.deviation
Group:			
Normal	11	6.7	6.3
Hyperdeploid	10	10.0	6.5
TEL/AML1 fusion gene	7	4.3	2.8
Other cytogen. abnorm.	18	9.7	7.3
Total[a]	46	8.2	6.6

[a]: p=0.195 (NS).

Table (5): Tolal Leucocytic Count (TLC) of patients with positive karyotype at presentation.

TLC	Case summaries		
	N	Mean	Std.deviation
Group:			
Normal	11	64.000	78.2573
Hyperdeploid	10	30.460	37.1892
TEL/AML1 fusion gene	7	39.600	66.0660
Other cytogen. abnorm.	18	61.272	96.6858
Total[a]	46	51.928	77.1209

[a]: p=0.699.

Table (6): Sex distribution of patients with positive karyotype at presentation.

	Sex			
	F		M	
	No	%	No	%
Group[a]:				
Normal	4	36.4	7	63.6
Hyperdeploid	6	60.0	4	40.0
TEL/AML1 fusion gene	3	42.9	4	57.1
Other cytogen. abnorm.	6	33.3	12	66.7

[a]: p=0.57 (NS).

Table (7): Post induction Status of 50 B lineage ALL patients with different cytogenetic findings.

Status	Total	Normal karyotype	High hyperdiploidy	TEL/ AML +	Others *
Complete remission	41/50 (82%)	9/11 (81.8%)	10/10 (100%)	7/7 (100%)	12/18 (66.6%)
No response	*9/50 (18%)	2/11 (18.2%)	0/10	0/7	6 (33.3%)
Mean overall survival (months)	21.1	22.8 p= 0.6337	26.9 p= 0.1030	28.3 p= 0.0757	15.0 p= 0.0134

*The 9th patient had failed karyotype.

DISCUSSION

Since the t(12;21) is virtually undetectable with conventional cytogenetic procedures, the two preferred screening methods are those with reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH). The latter technology has the advantage that it enables the identification and quantification of the most common and thus, most relevant secondary changes on a single cell level **[2,3,6,8, 33-35].**

The purpose of the present study was to identify the frequency of TEL/AML1 translocation among in comparison to other recurrent genetic abnormalities and to detect other abnormalities involving the TEL and AML1 genes either associated with or without the TEL/AML1 translocation in Egyptian children with B lineage ALL. We also aimed to demonstrate the usefulness of FISH in detecting such abnormalities. Finally we aimed to identify the correlation of such abnormalities with the overall survival.

A substantial increase in detection rate from 60% to 70% was observed using the combination of conventional G-banding and FISH analysis. Especially of note, FISH was useful to identify the cryptic gene rearrangements in cases with normal banded karyotype or in case of failure to obtain mitotic figures. A previously published Korean study reported successful CC in detecting chromosomal abnormalities in 49.2% of cases and when combining both CC and FISH the detection rate rose up to 73.8% **[42].**

The Children's Cancer Group (CCG) and the Pediatric Oncology Group (POG) agreed on that both Ph-positive ALL and extreme hypodiploidy were very poor risk factors and that TEL/AML1 fusion and triple trisomies of 4, 10 and 17 conveyed a very good prognosis **[34].**

In the current study, the frequency of TEL/AML1 translocation was found to be 14%. They all attained complete remission and have a mean overall survival of 28.3 months. Previously published studies reported the frequency of TEL/AML1 translocation to be around 25% in American, German, Italian and French populations **[34].** Two previous Egyptian studies reported the frequency to be 9.7% and 12.35% respectively **[35,36]** and others reported as 8.6% in

Indians **[37]** and in Japan 10% **[38]**. In Saudia Arabia the TEL/AML1 positivity was reported as 20% **[39]**. This difference may attribute to variability in inclusion criteria in the studies **[7, 8,33,34,40-41]**.

While in the present study, deletion of the non-translocated allele of TEL was detected in 2/7 cases of the TEL/AML1 gene fusion and a TEL deletion was found more often in the TEL/AML1+ cases than in the TEL/AML1- cases (28% vs 4.5%).

Similarly, another study detected 2/9 cases with TEL/AML1 fusion and simultaneous rearrangements of non-translocated TEL gene **[42]**. While other studies reported higher frequency of rearrangements of non-translocated TEL gene in more than 50% of the patients with TEL/AML1 fusion **[7,28]**. This supports the theory that the TEL/AML1 fusion gene acts in a recessive manner with regard to TEL gene, or that the secondary genetic changes including rearrangements of non-translocated TEL gene are needed in leukemogenesis by TEL/AML1 fusion **[11,15]**.

In the current study, the 2 cases with TEL/AML+ and TEL deletion achieved CR and are still in remission till the end of the study (35 and 29 months+) while others reported that one patient with non-translocated TEL deletion was classified into the high-risk group, whereas other patients with TEL/AML1 fusion alone were classified into low- or intermediate- risk group **[42]**.

Kempski et al. **[15]** did not find any difference in EFS between TEL-deleted (n=17) and non-TEL-deleted patients (n=5). On the contrary Attarbaschi et al. **[40]** studied 327 patients and found TEL/AML1 fusion in 94/327 (25%) and that cases with a TEL deletion had worse outcome than those without it.

In the current study, trisomy 21 was detected in TEL/AML1+ cases in 2/7 (28%) while in TEL/AML1- cases in 8/43 (18.5%). Others reported that the presence of trisomy 21 was not significantly different between the TEL/AML1+ and TEL/AML1- patients 13/94 (14%) vs 32/278 (12%) (p=0.785). The same study also detected no significant differences between TEL/AML1 patients with and without a trisomy 21 with respect to the presenting features and treatment outcome **[42]**.

In this work, AML1 gene amplification was detected as a sole chromosomal abnormality in one case which had a poor outcome. A previously published study identified 14 pediatric ALL cases with amplification or over-representation of 21q22 **[43]**. Another study reported that the amplification of AML1 is associated with a poor outcome **[18]**. A recently published study reviewed this novel cytogenetic finding (the intrachromosomal amplification of chromosome 21) which is typical for childhood B lineage ALL and requires high-risk therapy irrespective of other risk factors **[44]**.

In the present study, MLL gene rearrangement was detected in 3/50 (6%) A Korean study reported MLL gene rearrangement in 11.3% of cases for MLL **[42]**. Similar to our results, a Chinese study reported MLL gene rearrangement in 3/51 cases; one case showed t(4;11), another case showed MLL gene deletion as well as AML1 gene amplification as a large ring chromosome 21 and another case of MLL gene deletion together with t(12;21).

In the current study, BCR/ABL was detected in 3/50 cases (6%), one of them had double Philadelphia and hyperdiploid karyotype. Other studies reported 1.8% and 1.9% for BCR/ABL translocations **[42,45]**. Similar to one of our cases, a previously published study, reported a case of double Philadelphia chromosome, a hyperdiploid karyotype and duplication of chromosome 1 long arm **[45]**.

Among the TEL/AML1– patients included in this study, 14 cases showed either loss or gain of TEL and/or AML1 genes. Three cases showed TEL gene anomalies either deletion or trisomy and 10 cases with gain of AML1 gene either trisomy, tetrasomy or amplification. Our results agreed with what was published previously by Zang et al. **[45]**.

Finally, the current study confirms the well known important role of cytogenetics in risk stratification and outcome of pediatric ALL. Favorable outcome in childhood ALL includes hyperdiploidy, TEL/AML1 fusion versus unfavorable cytogentic abnormalities including the mixed lineage leukemia (MLL) and the translocation t(9;22) **[46-49]**.

In conclusion, TEL/AML1 gene fusion is a associated with a higher overall survival, similar

to high hyperdiploidy, than to normal karyotype and other chromosomal abnormalities such as BCR/ABL, MLL gene rearrangement, t(1;19), t(8;14) and hypodiploidy. Secondary chromosomal abnormalities are present in 4/7 of TEL/AML1+ cases. AML1 gene amplification is a less common chromosomal abnormality which is accompanied with poor outcome. Therefore, a larger scale study is recommended.

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