Research Article

Frequency of TEL-AML1 Fusion Gene in Patients of Acute Lymphoblastic Leukemia

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Abstract | Acute lymphoblastic leukemia (ALL) is the most common malignant tumor in children and forms a major fraction of childhood malignancies in the developed countries. Numerous chromosomal aberrations have been observed in ALL including translocations which result in the production of fusion genes (BCR-ABL, TEL-AML1, and MLL-AF4). These chromosomal rearrangements have great prognostic and therapeutic significance. Amongst these, *TEL-AML1* fusion gene represents a subgroup of ALL cases, which are associated with many clinically important parameters of good prognosis. The objective of this study was to detect the frequency of TEL-AML1 fusion oncogene in ALL and its association with already established prognostic factors such as age, WBC count and FAB subtype.

Materials and Methods: Sixty-six patients with newly diagnosed ALL were studied and patients on chemotherapy and T-ALL were excluded. The data was analyzed by SPSS20 for prognostically important parameters such as age, sex, hemoglobin level, WBC profile, platelet count, FAB type and immunophenotype. RNA extraction was performed and RT-PCR procedure was conducted to detect TEL-AML1 fusion oncogene.

Results: Out of 66 samples, frequency of TEL-AML1 fusion oncogene was detected in 5 subjects (7.6%). Almost all TEL-AML1 positive patients carried FAB ALL-L1, B-Lineage immunophenotype, mean hemoglobin level of 6g/dl and age from 3 to 5 years. All these features are related to good prognosis.

Conclusion: To conclude, we reported 7.6% frequency of TEL-AML1 fusion gene, in our study subjects, which is different from that reported in western literature. If universally accepted, the identification of TEL-AML1 fusion gene in ALL, will improve risk stratification and will help in selection of appropriate therapeutic regimens.

Introduction

Acute lymphoblastic leukaemia (ALL) is a frequently seen malignant disease of children.[1] ALL develops from the clonal proliferation and maturation arrest of lymphoid lineage cells in the bone marrow, blood and other organs.[2] Hereditary relations, genetic mutations, and possibly excessive exposureto ionizing radiations or chemicals are considered as the most potent etiologicalagents.[3] Many genetic alterations have been identified at the molecular level. [4] Of these, chromosomal abnormalities are found in

about 80-90 percent cases of childhood lymphoblastic leukemia.[5]

Chromosomal abnormalities in ALL may include hyperdiploidy with >50 chromosomes, hypodiploidy with <44 chromosomes, and chromosomal translocations e.g. t (12;21) *TEL-AML1*, t(1;19) *E2A-PBX1*, t(9;22) *BCR-ABL1*, t(4;11) *MLL/AF4*, t(5;14) *IL3-IGH*. The presence of definitive chromosomal aberrations in patients of ALL is one of the most important prognostic parameters. Therefore, the recognition of these cytogenetic abnormalities is useful to identify prognostically relevant subtypes of ALL.[6]

TEL-AML1 chimeric gene is found in almost 25% of ALL patients.[7] *TEL-AML1* fusion transcript positive ALL cells have much greater levels of pro-apoptotic protein Fas and decreased levels of anti-apoptotic protein Bcl2 than their negative counterparts. *TEL-AML1*chimeric gene positive ALL cells are extremely sensitive to vincristine, dexamethasone and apoptotic inducing effects of serum deprivation [8] pointing towards good prognosis of this subtype of ALL.[9] With advancing age, the incidence of molecular abnormalities associated with good prognosis such as *TEL-AML-1* decreases and abnormalities associated with poor prognosis such as *BCR-ABL-1*are increased.[10]

Keeping in view the importance of *TEL-AML1* fusion gene linked to prognosis and also risk assessment, this study was designed to determine the frequency of *TEL-AML 1* fusion gene in patients of acute lymphoblastic leukaemia and to study its association with known prognostic laboratory and clinical factors such as age, WBC count, and FAB subtype.

Materials and Methods

A descriptive study was conducted in the Department of Hematology, University of Health Sciences, Lahore, Pakistan. The study was approved by the local ethical committee. Patients were enrolled after informed written consent from parents/guardians. Sixty-six patients of all age groups and of both genders with diagnosis of ALL were selected. Diagnosis of ALL was conduced on the basis of morphological features, cytochemical stains and Immunophenotyping while ALL patients on chemotherapy, T-ALL or with relapse of ALL were excluded from study. Patients were selected from children hospital Lahore,

Jinnah Hospital Lahore and Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan. All demographic and clinical features including age, diagnosis, morphology were recorded for each patient as well as clinical history.

After ensuring aseptic measures, a total of 5ml of blood from peripheral vein or 1ml of bone marrow aspirate was taken in EDTA vaccutainers. The CBC was performed on Sysmex XT 1800i. Smears were prepared and stained with Giemsa staining for morphology and the rest of sample was stored in EDTA vaccutainers for RNA extraction. RNA extraction was conducted within two to three hours post-sample collection. After extraction of RNA, it was stored at -80 °C.

Total RNA extracted was reverse-transcribed to cDNA by using it as a template in RT-PCR reaction. The reverse transcription reaction was catalyzed by enzyme Reverse Transcriptase (RT). About 0.1ng -5 μg of patient RNA was reversely transcribed into cDNA by using "Thermo scientific revertaid first strand cDNA synthesis kit". The cDNA was synthesized by using specific primers in a total volume of 20μl and then stored at -20°C. This first strand cDNA was used in control PCR amplification in presence of forward and reverse GAPDH primers. From this PCR reaction, 5-10 μl was loaded on 2% agarose gel. A distinct 453 bp PCR product was visible after ethidium bromide staining.

After cDNA synthesis, reverse transcriptase polymerase chain reaction (RT-PCR) was employed for detection of *TEL-AML1* chimeric transcript derived from translocation t(12;21). Nested primers were used for this RT-PCR reaction, thereby yielding maximum sensitivity and specificity. Sequences of these nested primers were taken from that used byLin.[5]

Nested RT-PCR was performed in two major rounds. The first round of the nested RT-PCR was carried out using external primers and the second one using internal primers. PCR amplifications were carried out using complementary DNA (cDNA) in a thermocycler applying a nested RT-PCR primer approach (35 cycles each time). Two μl was added from control RT-reaction to 48μl of reaction master mixture carefully (the reaction master mixture for first round of nested PCR consisted of 10X PCR Buff-

er 5μl, 10mMdNTP 1μl, 25mM Mgcl$_2$ 3μl, 1.5μl of each primer pair (external primers) and *taq* DNA polymerase 0.5μl (5U/μl) and 35.5μl of nuclease free water to make volume to 50μl. The initiation of PCR process comprised of polymerase enzyme activation at 94ºC for 3minutes, followed by 35 cycles of PCR amplification (denaturation at 94ºC for 45seconds, annealing at 58ºC for 45seconds, elongation at 72ºC for 45 seconds). After completion of the first round of nested type of RT-PCR, 1μl from each of these first round amplifications were transferred to second round reactions, which were similar to the first round reactions with the exception that internal primers were added in place of external primers. The final products were visualized by gel electrophoresis. A distinct 228bp PCR product was visible after ethidium bromide staining. All necessary measures were taken to avoid contamination. A negative control and a positive control were added in each amplification cycle.

When compared with 50base pairs control ladder on 2 % agarose gel, stained with ethidium bromide, TEL-AML1 fusion gene product size was found to be 181 base pairs.

Results

In this study, 101 patients were selected who were initially diagnosed as cases of acute lymphoblastic leukemia on the basis of morphology and cytochemical stains. The follow up data of fifteen patients showed T-ALL on immunophenotyping. These fifteen patients were excluded from the study. Only those patients were selected who showed B-ALL immunophenotype. Further twenty patients were excluded because of insufficient quantity of RNA in their samples and no cDNA was detected on gel electrophoresis, and remaining sixty six patients wereanalyzed for the presence or absence of *TEL-AML1*chimericgene.

Most of our patients presented with pallor and high-grade fever for more than a month of duration. Lymphadenopathy, splenomegaly and hepatomegaly was observed in most of the patients with variable proportions.

There was no patient less than one year of age in this study. Forty-eight (73%) subjects were between one to ten years and eighteen (27%) were above ten years. Mean age at presentation was 10.76 ± 12.87 years and range was 2-70 years. Forty (60.6%) were male and 26

(39.4%) were female. Mean Hb was 8.36±2.4, mean WBC count 54.29±101.6 while mean platelet count was 60.21±62.38 at presentation (Table 1).

Table 1: *Demographic features of patients (n=66), selected in the study.*

Features	Groups	Frequency	Percentage (%)
Age	<1	0	00
	1-10	49	74
	>10	17	26
Gender	Male	40	60.6
	Female	26	39.4
WBC (10⁹/L)	<50	45	68.2
	50-100	12	18.2
	>100	09	13.6
Platelet count (10⁹/L)	<100	57	86.4
	>100	09	13.6
Hemoglobin (g/dl)	<6	09	13.6
	6-9	33	50
	>9	24	36.4

Data was given in frequency and percentage

Lymphadenopathy was present in 40 (60.6%) patients while hepatomegaly was present in 43 (65.2%) and absent in 23 (34.8%) patients. Splenomegaly was found in 55 patients. Fifty four patients had ALL-L1 while 12 had ALL-L2.Most of the patients 51 (77.8%) were of pre-B and a small number 15 (22.2%) were of precursor-B immunophenotype.

Out of sixty-six patients, sixty one (92.4%) were TEL-AML1 fusion gene negative and five (7.6%) were positive (Figure 1).
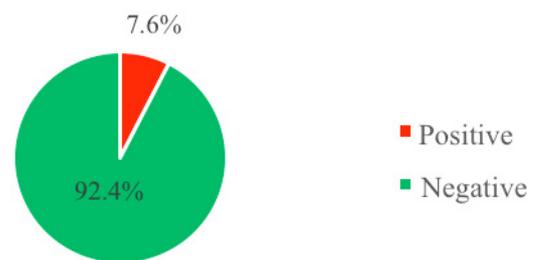

Figure 1: *Frequency of TEL-AML1 fusion gene, Red; TEL-AML1 positive (7.6%), Green; TEL-AML1 negative (92.4%).*

Of five *TEL-AML-1* positive patients, four were male and one was female. All were with age ranging between 3-5 years. Hemoglobin level was in range of 4.1-9.2g/dl with mean value of 6g/dl. Total leucocyte count (TLC) was in the range of 12.4-488.7 x10⁹/L while range of platelet was found to be 13-193 x10⁹/L. Lymphadenopathy was present in only one of five

TEL–AML–1 positive patients, whereas hepatomegaly and splenomegaly was present in all five *TEL–AML1* positive patients. All of five *TEL–AML–1* positive patients were with FAB type, ALL-L1 and were with Pre-B immunophenotype (Table 2).

TEL-AML1 positive patients were mainly males, age varied from 3 to 5 years. All were having FAB type ALL-L1 and B-Lineage immunophenotype. Hemoglobin level was in range of 4.1-9.2 g/dl while white blood cells count was in range of 50-100 x10^9/L (Table 3).

Discussion

Genetic aberrations mainly translocations are found in more than 75% cases of acute lymphoblastic leukemia (ALL). Finding genetic aberrations are prerequisites for risk stratification to adopt risk directed treatment strategies.[11] TEL-AML1 fusion gene is

Table 2: *Demographic, laboratory and clinical features of TEL–AML1 positive patients*

S.no.	Sex	Age	Hb g/dl	TLC 10^9/L	Platelet count10^9/L	Lymphadeno-Pathy	Hepato-Megaly	Spleno-Megaly	FAB type	Immuno-phe-notype
1	M	04	4.1	97.8	193	Present	Present	Present	L1	Pre-B
2	F	03	9.2	66.7	13	Absent	Present	Present	L1	Pre-B
3	M	04	4.7	88.6	61	Absent	Present	Present	L1	Pre-B
4	M	04	4.7	488.7	29	Absent	Present	Present	L1	Pre-B
5	M	05	7.3	12.4	90	Present	Present	Present	L1	Pre-B

Table 3: *Comparison of clinical and laboratory features of TEL–AML1 positive and negative patients.*

Features / parameters		TEL-AML1 positive (n=5)		TEL-AML1 Negative(n=61)	
		Frequency	Percentage (%)	Frequency	Percentage (%)
Gender	Male	04	80	36	69
	Female	01	20	25	31
Age (years)	<1	00	00	00	00
	1-10	05	100	44	72.1
	>10	00	00	17	27.9
Hemoglobin (g/dl)	<6	03	60	06	9.8
	6-9	01	20	32	52.5
	>9	01	20	26	37.7
TLC (x10^9/L)	<50	01	20	44	72.1
	50-100	03	60	09	14.8
	>100	01	20	08	13.1
Platelet count (x10^9/L)	<100	04	80	53	86.9
	>100	01	20	08	13.1
Lymphadenopathy	Present	02	40	38	62.3
	Absent	03	60	23	37.7
Hepatomegaly	Present	05	100	38	62.3
	Absent	00	00	23	37.7
Splenomegaly	Present	05	100	50	82
	Absent	00	00	11	18
FAB Type	ALL-L1	05	100	49	80.3
	ALL-L2	00	00	12	19.7
Immunophenotypes	Pre- B	05	100	46	75.4
	Precursor B	00	00	15	24.6

Data given in frequency and percentage

Links Researchers

the most frequent genetic aberration found in B-lineage ALL cases in western countries.[12] TEL-AML-1 positive ALL cells are extremely sensitive to chemotherapy, indicating better prognostic subtype of ALL.[8] Because of geographic and racial differences, the prevalence of different fusion genes might vary between west and south-east Asian countries including Pakistan. The purposeof present study was to the assess the TEL-AML1 fusion gene status in our population andalso to analyze its correlation with already established prognostic factors.

In this study,out of 66 patients, males were forty whereas females were 26. While regarding gender of five TEL-AML1 positive patients, four patients (80%) were male and one (20%) was female. This is in accordance with previous study conducted by Heba M Shaker.[13] In his study, seven of the 10 TEL-AML1 positive patients were males (70%) and three were females (30%). ALL is seen in both children and adults, but its incidence peaks between two to five years of age.[14] In the current study 48 (73%) patients were equal to or less than ten years of age. Mean age at presentation was 10.76±12.86. Clinical and laboratory features with recognized prognostic value in ALL include age, sex, initial total leukocyte count, degree of organomegaly and early response to therapy as well as immunophenotype. Patient age and leukocyte count at diagnosis provide the most important prognostic information.[13] It has also been reported that frequency of TEL-AML1 fusion gene is maximum within age range of one to ten years.[9] All our TEL-AML1 positive patients were between three to six years of age. Our results confirm the reports on clinical features of TEL-AML1 positive cases, thus assigning our TEL-AML1 positive patients to the standard risk group.[9,13]

In the present study, hepatosplenomegaly was the most common finding at presentation. Hepatomegaly was present in 43 patients (65.2%) whereas splenomegaly was present in 55 patients (83.3%). Regarding organomegaly, our results are consistent with other studies.[6,11] In the present study lymphadenopathy was present in only one of five TEL-AML1 positive patients, whereas hepatomegaly and splenomegaly was present in all five TEL-AML1 positive patients. Faiz and coworkers reported similar findings in their study.[6]

Mean WBC at presentation was 54.29±101.64 x10⁹/L

whereas count> 100x 10⁹/L was observed in 9(13.6%) cases as also suggested by Pandita and coworkers.[11] The total white cell count of one of five TEL-AML1 positive patients was less than 50 x 10⁹/L, three were within the range of 50-100 x 10⁹/L and one was with exceptionally high WBC count of 488.7 x 10⁹/L. Mean WBC count of TEL-AML1 positive cases was high i-e 150.84 x10⁹/L at presentation. Regarding WBC count our results are contrary to that observed by Heba M. Shaker and co workers. In their study, five (71%) out of seven TEL-AML1 positive patients were having WBC count less than 50 x 10⁹/L, in two patients (29%) WBC count was within range of 50-100 x 10⁹/L and none of their patients presented with WBC count of >100 x 10⁹/L.[13] Other studies also suggest similar WBC counts i-e ≤ 50 x 10⁹/Lin most of the TEL-AML1 positive patients.[5,9]

Mean hemoglobin level at presentation was 8.362±2.4 g/dl. Most of the patients (50%) were having Hb level of 6-9 g/dl while in twenty-four (33.4%) patients; Hb was more than 9 g/dl. Settin and coworkers reported similar findings in their study.They found that 41 of 63 (65%) patients were with hemoglobin equal to or less than 8g/dl and 22 (35%) were with hemoglobin level of more than 8g/dl.[1]Our TEL-AML1 positive patients had hemoglobin levels ranging from 4.1-9.2g/dl with mean value of 6g/dl. Regarding Hb levels in TEL-AML1 positive cases, our results are consistent with another research in which mean Hb was 5.75g/dl and range was 5-7.5g/dl in TEL-AML1 positive cases.[11]

The FAB type, and ALL-L1 were found in most of our patients. Out of 66, 54 cases (81.8%) were having ALL-L1 and a small number 12(18.8%) had ALL-L2. In previous studies FAB type, ALL-L1was found in majority of ALL patients.[6,11] All of five TEL-AML1 positive patients were with FAB type, ALL-L1. Our finding is consistent with the study of Pandita and coworkers who found that three out of eightTEL-AML1 positive patients were havingFAB type; ALL-L1 whereas only one was with ALL-L2.[11] The immunophenotype of all TEL-AML1 positive patients was Pre-B. This is in accordance with a previous study in which six out of 10 TEL-AML1 positive patients were having Pre-B cellimmunophenotype[6] but different from a research conducted in Taiwan in which half (50%) of the TEL-AML1 positive cases were having precursor-B immunophenotype.[5]Again, this immunophenoype places our TEL-AML1 pos-

itive cases in the standard risk group regarding response to therapy and incidence of relapse.

In current study, Five (7.6 %) of sixty six patients were positive for TEL-AML1 fusion gene. The results of the present study are in accordance with previous studies conducted in Pakistan and India.[6,15] In these studies, the frequency of TEL-AML1 fusion gene was found to be 9.7% and 8.69%, respectively. Our results are contrary to that observed by other researchers in Taiwan and USA,[5,9] in which higher frequency of TEL-AML1 i-e 32% and 26% was found. Another study has reported 00% frequency of TEL/AML1 fusion gene transcript in Spain.[16]

International differences in incidence rates of TEL-AML1 fusion gene transcript are related with genetic and/or environmental factors that might contribute to pathogenesis of the ALL. Keeping in view, already published data from Pakistan and India, the results of current study are almost close to previous studies. Possible reason for this closeness can be that the people of both countries living across the border share almost similar dietary habits, common environmental conditions and same lifestyle. Therefore, it is required to tailor our protocols accordingly for the treatment of ALL patients.

Conclusion

In conclusion, frequency of TEL-AML1 fusion gene was found to be 7.6% in our ALL cases. Positive cases carry features for good prognosis, namely the 3-5 year age group, B-Lineage immunophenotype, mean hemoglobin level of 6 g/dl and FAB type, ALL-L1. TELAML1 positive patients can thus be considered a distinct clinical entity which deserves thorough molecular screening and long-term prospective clinical trials to further clarify the prognostic impact of this fusion and accordingly design treatment protocols with the least deleterious effects on the growth and development of children suffering from ALL.

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Author's Contribution

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