RESEARCH COMMUNICATION

Does TAL-1 Deletion Contribute to the High Incidence of T-Cell Acute Lymphoblastic Leukemia in South Indian Patients?

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Abstract

Background: The incidence of T-cell acute lymphoblastic leukemia (T-ALL) in South India is very high (43.1%) when compared to the Western countries (10-20%). *TAL-1* deletion is the most common genetic abnormality in T-ALL. Objectives: The present study was aimed to detect the incidence of type 1 and type 2 *TAL-1* deletions and assess whether they might contribute to the high incidence of T-ALL in South India. Materials and Methods: 45 cases of T-ALL (pediatric-32, adolescents-7 and young adults-6) were studied by DNA-PCR and sequencing. Age of the patients ranged from 3 yrs to 29 yrs (median age 14 yrs). Results: *TAL-1* deletion type 1 was detected in 6 (13.3%) cases (3 pediatric and 3 adolescents) and all were males. *TAL-1* deletion type 2 was not present. Comparing the clinical features and immunological marker analysis of *TAL-1* deletion positive and negative cases did not show any significant differences except in the WBC count, which was significantly higher in cases showing *TAL-1* deletion (>100 x 10^9/L, p value= 0.003). All the positive cases of *TAL-1* deletion were confirmed by sequencing, the results showing that the fusion region at *SIL* gene and *TAL-1* gene contained an average 'N region' insertion of 7.8 nucleotides. The numbers of nucleotides deleted at the 5' end and 3' end of *TAL-1* gene were averages of 3 and 1, respectively. Conclusion: Though the incidence of T-ALL is high in South India, the frequency of *TAL-1* deletion and their fusion gene sequences are not unique and are similar to those reported in other ethnic and geographic populations. Hence the present study indicates that *TAL-1* deletion alone does not contribute to the high incidence of T-ALL in South Indian patients.

Key Words: *TAL-1* deletions - DNA-PCR - sequencing - fusion region

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Introduction

T-cell acute lymphoblastic leukemia (T-ALL) occurs by the uncontrolled proliferation of T-lymphoid precursors arrested during distinct stages of differentiation. The percentage of T-ALL in South India is very high (43.1%) when compared to the Western countries (15-25%) (Rajalekshmy et al., 1997; Brunning et al., 2001). *TAL-1* deletion is the most common genetic abnormality in T-ALL. The *TAL-1* gene, also known as *SCL or TCL5* located on 1p32, encodes a basic helix loop helix protein Tal-1 (Aplan et al, 1990; Begley et al, 1990) and is essential for the earliest stages of hematopoietic stem cell development and differentiation (Begley et al., 1990; Porcher et al., 1996).

Translocation of *TAL-1* (1p32) next to the TCRd loci in the t(1;14) (p32:q11), occurs in 3% of T-cell ALL and results in aberrant *TAL-1* expression (Carroll et al., 1990). A frequent mode of *TAL-1* deregulation is a site-specific deletion (*TAL-1* deletion) of ~90kb as a result the coding exons of the *TAL-1* gene are juxtaposed to the first non-coding exon of the *SIL* gene, which is almost completely deleted (Brown et al, 1990). The expressed *SIL-TAL1* fusion transcript produces a normal Tal-1 protein, but it is transcriptionally controlled by the *SIL* gene promoter (Bernard et al., 1991; Aplan et al., 1992).

TAL-1 deletions are not detected by classical cytogenetics. *SIL* contains three donor deletion sites (Sildb1 to Sildb3), of which Sildb1 is the most commonly used (98% of cases). *TAL-1* contains seven acceptor deletion sites (taldb1 to taldb7), with two being involved in almost all cases (taldb1 and taldb2). The vast majority (~90%) of *TAL-1* deletions are located between Sildb1 and taldb1 and are known as *TAL-1* deletion type I. The *TAL-1* deletion type 2 occurs between Sildb1 and taldb2 (Breit et al, 1993). The present study was aimed to detect the incidence of type 1 and type 2 *TAL-1* deletions, their fusion gene sequences and assess whether *TAL-1* deletion contributes to the high incidence of T-ALL in South Indian patients.

Patients and Methods

Patients

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T-ALL patients presenting to Cancer Institute (WIA) were included in the present study. Bone marrow (BM) and peripheral blood (PB) samples were obtained from 45 T-ALL patients (median age 14) prior to starting the treatment. Among these cases, 32 were pediatric, 7 were adolescents and 6 were young adults. All the patients were enrolled for treatment after a written Informed consent.

Collection of BM and PB and Separation of Mononuclear Cells

Two milliliters of BM and 10 ml of PB were collected from the patients. The mononuclear cells (MNC) were separated using Ficoll HypaqueTM (density: 1.077g/ml, Amersham Biosciences, Uppsala, Swedan) density gradient centrifugation. The cells were washed twice with PBS and stored at -70°C until use.

DNA Isolation From MNC

DNA was isolated from the MNC using QIAamp kit (Qiagen, Hilden, Germany) and quantitated by Gene Quant (Amersham); the quality was checked by amplifying for the *C-ABL* housekeeping gene.

PCR for Type 1 and Type 2 TAL-1 deletion

PCR was performed in a 25µl reaction containing 10X PCR buffer, 1.5mM $MgCl_2$, 200µM of dNTP's (AB Gene, UK), 1U of Hotstart Taq Polymerase (AB Gene, UK), 10 pmol of forward and reverse primers with 100ng of genomic DNA. PCR was performed using a Perkin-Elmer 480 thermal cycler (Applied Biosystems). PCR conditions included preactivation of the enzyme for 10 min at 94°C followed by 35 cycles for 94°C for 60s, 60°C for 90s and 72°C for 2 min and then a final extension of 10 min at 72°C. The primers used for *C-ABL* gene were 5' GGCCAGTAGCATCTGACTTTG 3' (forward primer) and 5' ATGGTAC CAGGA GTGT TT CTCC 3' (reverse primer). The primers for detecting *TAL-1* deletions are Sildb1 5' AAAGAGGG TGATC GAGGGGAA 3' (forward primer) and reverse primers 5' AGAGCCTGTCGCCAAGAA 3' (for *TAL-1* deletion type 1) and 5' TTGTAAAATGGGGAGATAATGTCGAC 3' (for *TAL-1* deletion type 2) (Pongers-Willemse et al, 1999). A positive control (CEM cell line for type 1 *TAL-1* deletion) and negative control (water instead of DNA) were included in all PCR experiments. Amplified products were visualized by electrophoresing on a 3% agarose gel.

DNA Sequencing

TAL-1 deletion type 1 amplified PCR products were directly sequenced with forward and reverse primers. For sequencing, the Big Dye Terminator Cycle Sequencing Ready Reaction kit v3.0 (Applied Biosystems, Foster City, USA) was used and the reaction products were analyzed in a ABI 310 Genetic analyzer.

Statistical analysis

Fisher's exact test in a 2x2 table was used to compare the clinical features of *TAL-1* deletion positive cases and *TAL-1* deletion negative cases. A p value of <0.05 was considered as statistically significant.

Table 1. Clinical Features and Immunological Markers in *TAL-1* Deletion Positive and Negative Cases

Clinical features	*TAL-1* del positive cases (n=6)	*TAL-1* del negative cases (n=39)
Sex		
Male	6 (100)	28 (71.7)
Female	0 (0)	11 (28.2)
Age		
Range	7 yrs to 17 yrs	3 yrs to 29 yrs
Median	14 yrs	14 yrs
WBC count (10^9/L)		
<20	0 (0)	13 (33.3)
20-50	0 (0)	8 (20.5)
50-100	0 (0)	5 (12.8)
>100	6 (100)	13 (33.3)
Immunological markers		
CD2	6 (100)	29 (74.3)
CD3	2 (33.3)	23 (58.9)
CD5	6 (100)	34 (87.1)
CD7	4 (66.6)	39 (100)
CD4	1 (16.6)	14 (35.8)
CD8	3 (50)	20 (51.2)
Generalized lymph node -enlargement	3 (50)	24 (61.5)
Hepatosplenomegaly	2 (33.3)	22 (56.4)
Mediastinal mass	1 (16.6)	11 (28.2)

Results

A total of 45 T-ALL cases were studied for *TAL-1* deletion type 1 and type 2 by DNA-PCR. The quality of the DNA was checked by amplifying for *C-ABL* house keeping gene. The DNA was then used for detecting type 1 and type 2 *TAL-1* deletion. *TAL-1* deletion type 1 was detected in 6 (13.3%) cases. *TAL-1* deletion type 2 was not present in these cases. The type 1 *TAL-1* deletion amplified products of size 330bp is shown in Figure 1a.

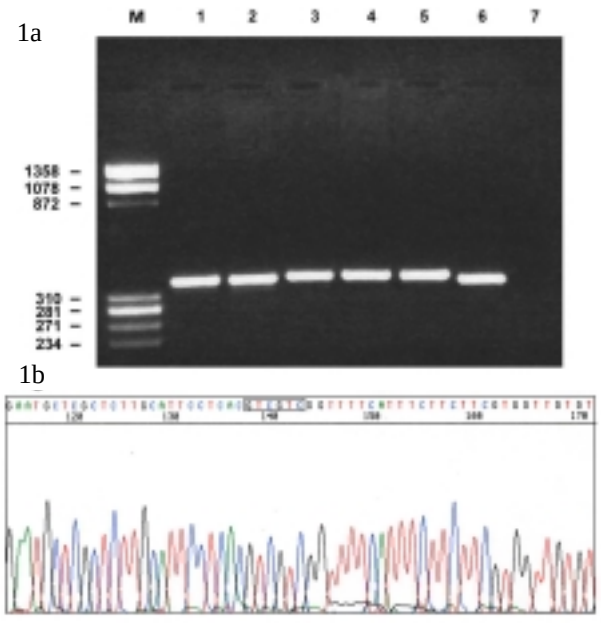

Figure 1a. PCR for Type1 *TAL-1* deletions. Lanes 1 to 6 shows amplified products of Type1 *TAL-1* deletion (330bp); lane 7- negative control. Figure 1b. Sequencing of Type1 *TAL-1* deletion. 5' *TAL-1* deletion Sequence, 3' *TAL-1* deletion sequence and fusion region (GTCGTC) of a T-ALL case is shown

Table 2. Fusion Gene Sequences of *TAL-1* Deletion (type 1) Cases

Pt code	5' *TAL-1* deletion	Fusion region	3' *TAL-1* deletion
Germline sequence	TCCTCACAATTTCTGGCTCT		GTTGGTTTTCATTTCTTCTT
1	TCCTCACAATTTCTGGCTC	TGAATGAACTCC	GTTGGTTTTCATTTCTTCTT
2	TCCTCACAATTTCTGGCTC	GGG	GTTGGTTTTCATTTCTTCTT
3	TCCTCAC	CCG	GTTGGTTTTCATTTCTTCTT
4	TCCTCACAATTTCTGGCTC	GGGATCCGGAGCGA	GGTTTTCATTTCTTCTT
5	TCCTCACAATTTCTGGCTCA	TGGGGGAAC	GTTGGTTTTCATTTCTTCTT
6	TCCTCAC	GTCGTC	GGTTTTCATTTCTTCTT
CEM (Positive control)	TCCTCACAATTTCTGGCTC	AAGTGGA	GTTGGTTTTCATTTCTTCTT

All the cases showing *TAL-1* deletion type 1 were males with significantly high WBC count >100 × 109/L (p value= 0.003). The clinical characteristics and immunological marker analysis of *TAL-1* deletion positive and *TAL-1* deletion negative cases are shown in Table 1. In cases showing *TAL-1* deletion, CD2 and CD5 were detected in all the positive cases, surface membrane CD3 was detected in 2 cases, CD4 was detected in one case, CD7 was detected in 4 cases and CD8 was detected in 3 cases.

Sequencing was done in samples that showed *TAL-1* deletion type 1. The 5' and 3' *TAL-1* deletion and fusion region sequences are shown in Table 2. The fusion region contained an average of 7.8 nucleotides. The number of nucleotides deleted at the 5' end and 3' end on an average was 3 nucleotides and 1 nucleotide respectively. The fusion region of *SIL* and *TAL-1* gene is shown in Figure 1b.

Discussion

The reason for the high incidence of T-ALL in South Indian patients remains unknown. We have undertaken this study to detect the incidence of *TAL-1* deletion and assess whether it contributes to the high incidence of T-ALL in South Indian patients. In the present study, *TAL-1* deletion type 1 was detected in 6 (13.3%) cases. Among these cases, 3 were pediatric and 3 were adolescents. This is in line with the observations that *TAL-1* deletion is less frequent in adult ALL (Asnafi et al, 2004). Brown et al reported 26% of type 1 *TAL-1* deletions in their study (Brown et al, 1990). Breit et al in 134 T-ALL cases reported 4 types of *TAL-1* deletion with 10.4% of type 1 and 2.2% of type 2 *TAL-1* deletion (Breit et al, 1993). *TAL-1* deletion type 2 was not detected in the present study. The absence of type 2 *TAL-1* deletion in this study would have occurred due to the small number of cases.

Comparing the clinical features of cases showing *TAL-1* deletion positive and *TAL-1* deletion negative revealed WBC count as the only significant variable between the two groups. The expression of T-cell markers CD3, CD4 and CD7 were less in cases showing *TAL-1* deletion compared to *TAL-1* deletion negative cases but they have not reached statistical significance. The patients with *TAL-1* rearrangements at presentation are usually associated with high WBC count, CNS disease, immunophenotype of CD2+, CD5+ and CD7- and CD3- (Aplan et al., 1992). All the cases showing *TAL-1* deletion in the present study were males. It needs to be verified that *TAL-1* deletion is more associated with male gender or occurred by chance due to the preponderance of males in South Indian T-ALL patients.

The *TAL-1* fusion region consisted of an average insertion with 7.8 nucleotides. A study by Breit et al showed an average N-region insertion of 6.4 nucleotides in *TAL-1* fusion region (Breit et al, 1993). The present study has showed the frequency of type 1 and type 2 *TAL-1* deletions and information on *TAL-1* fusion region in South Indian T-ALL patients. In conclusion, though the incidence of T-ALL is more in South India, the frequency of *TAL-1* deletion and their fusion gene sequences are not distinct and are similar to that reported in other ethnic and geographic populations. Hence, *TAL-1* deletion alone does not contribute to the high incidence of T-ALL in South Indian patients. Also, the present study makes it necessary to study other molecular abnormalities in T-ALL such as $p16^{INK4a}$ deletion, *Notch1* gene mutation and *TLX1 (HOX11)* gene expression to assess whether they contribute to the high incidence of T-ALL in South Indian Acute Lymphoblastic Leukemia patients.

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