The Libyan Journal of Science (An International Journal): Volume 19B, 2016

Prevalence of Mutations in *TAL1* Gene in Individuals With T-ALL and T-NHL

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Abstract

Mutations in the *TAL1* (T-cell acute leukemia 1) gene were recently described in patients with T-cell acute lymphoblastic leukaemia (T-ALL) and in those with lymphoblastic T-cell non-Hodgkin's lymphoma (T-NHL). The purpose of this pilot study was to assess the prevalence of mutations in *TAL1* gene in T-ALL and T-NHL. DNA samples from 15 unrelated healthy controls, 20 T-ALL patients, and 10 T-NHL patients were analyzed using DNA-PCR and direct DNA sequencing to identify sequence genetic variations in *TAL1* gene (exons 2 and 3).

TAL1 exon 2 mutations were identified in 7.7% adult and 12.5% adolescent T-ALL patients analyzed. *TAL1* exon 2 mutations were detected in 16.7% of the adult T-NHL patients analyzed. Sequencing of *TAL1* exon 3 showed no sequence variation for the T-ALL and T-NHL cancer patients analyzed. No sex difference where observed in the incidences of *TAL1* exons 2 mutations between T-ALL and T-NHL patients with and without *TAL1* mutations. *TAL1* exon 2 missense and frame-shift mutations were present in 44.4% (4/9) and 55.6% (5/9) of T-ALL patients, respectively. However, the frame-shift and missense mutations in the T-NHL patients accounted for, where respectively, 60% (3/5) and 40% (4/5) of all *TAL1* exon 2 mutations.

Comparing the clinical features showed that there are no differences in PLT and WBC counts as well as the average age between T-ALL and T-NHL patients with and without *TAL1* mutations. Overall, these findings indicate that *TAL1* mutations are too rare to be of clinical relevance, and do not seem to be significantly associated with the increased T-ALL and T-NHL susceptibility, implying different pathways with respect to *TAL1* genetic polymorphisms as a risk factor for T-ALL and T-NHL at least in this population of Libyans.

Keywords: T-cell acute lymphoblastic leukaemia (T-ALL); T-cell non-Hodgkin's lymphoma (T-NHL); T-cell acute leukemia 1 (*TAL1*) gene; mutation; susceptibility.

Accepted for publication: 8/12/2016

المستخلص

أتبتت الدراسات الحديثة أن وجود طفرات في مورثة TAL1 مرتبطة بمرضي سرطان T-ALL و T-NHL. هدفت هذه الدراسة إلى تقييم مدى انتشار الطفرات في مورثة TAL1 في مرضي T-ALL و T-NHL. تم تجميع عدد 15 عينة من الحمض النووي DNA من أشخاص أصحاء، و20 عينة من مرضى T-ALL و10 عينات من مرضى T-NHL وتحليل المادة الوراثية باستخدام تقنية DNA-PCR و Sequencing DNA وذلك لتحديد التغيرات الوراثية في تتابعات الأكسون 2 و3 من مورثة TAL-1.

أوضحت نتائج هذه الدراسة وجود طفرات في الأكسون 2 في 7.7% من البالغين و12.5% من المراهقين من مرضي T-ALL. أظهرت النتائج أيضا وجود الطفرات في الأكسون 2 في حوالي 16.7% من البالغين المصابين بمرض T-NHL. لم تظهر نتائج هذه الدراسة وجود طفرات في الأكسون 3 من نفس المورثة في كلاً من مرضى T-ALL و T-NHL. كما لم تظهر النتائج وجود فروق معنوية بين الجنسين من حيث وجود طفرات في الأكسون 2 للمورثة TAL-1 بين مرضى T-ALL ومرضى T-NHL. الطفرات المغلوطة وطفرات الإزاحة في الأكسون 2 للمورثة TAL-1 كانت تمثل 44.4% و 55.6% على التوالي في مرضى T-ALL. شكلت الطفرات المغلوطة والإزاحة في مرضى T-NHL حوالي 60% و40% على التوالي من الطفرات في الأكسون 2. أظهرت النتائج السريرية عدم وجود اختلاف معنوي في أعداد كلاً من الصفائح الدموية و كرات الدم البيضاء، فضلاً عن متوسط العمر بين مرضي T-ALL ومرضى T-NHL سواء عند أولئك الذين ليس لديهم أو لديهم طفرات في الأكسون 2 لمورثة TAL-1.

تشير نتائج هذه الدراسة إلى أن الطفرات في مورثة TAL-1 ليست ذات أهمية طبية، ولا يبدو أنها تترافق مع زيادة القابلية للإصابة بمرض T-ALL ومرض T-NHL، مما يدل على وجود مسارات أخرى تتعلق بخطر الإصابة بمرض T-ALL ومرض T-NHL على الأقل بالنسبة لهذه العينة من المرضى الليبيين.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) and lymphoblastic T-cell non-Hodgkin's lymphoma (T-NHL) are closely related disorders and although T-ALL cells are more often similar to the early stages of thymocytes than T-NHL cells, there is a considerable overlap between these two disorders [1-2]. Morphology, ultrastructure and immunocytochemistry do not allow us to distinguish them and even clinically, a clear cut discrimination between T-ALL and T-NHL is not evident [3]. In fact, T-NHL is usually differentiated from T-ALL by minimal or absence of bone marrow and peripheral blood involvement, normal white blood

cell (WBC) count, normal hemoglobin levels and lack of organomegaly. Because this distinction does not apply in every case, a presence of less than 25% of blasts in the bone marrow is currently used to define T-NHL from T-ALL [4]. T-ALL occurs by the uncontrolled proliferation of T-lymphoid precursors arrested during distinct stages of differentiation [5]. The percentage of T-ALL in India is very high (43.1%) when compared to the Western countries (15-25%) [6]. *TAL-1* deletion is the most common genetic abnormality in T-ALL [5].

The T-Cell Acute Lymphocytic Leukemia 1 (*TAL1)* gene (also known as *SCL* or *TCL5*) located on 1p32, encodes a basic helix loop helix protein *TAL-1* [7] and is essential for the earliest stages of hematopoietic stem cell development and differentiation [7]. Translocation of *TAL1* (1p32) next to the TCRd loci in the t(1;14) (p32:q11), occurs in 3% of T-cell ALL and results in aberrant *TAL1* expression [5]. A frequent mode of *TAL1* deregulation is a site-specific deletion (*TAL1* deletion) of ~ 90 kb. As a result the coding exons of the *TAL1* gene are juxtaposed to the first non-coding exon of the *SIL* gene, which is almost completely deleted [8]. The expressed *SIL-TAL1* fusion transcript produces a normal *TAL1* protein, but it is transcriptionally controlled by the *SIL* gene promoter [9]. Cytogenetic investigations of large NHL series reported abnormal karyotypes, often with complex abnormalities, in about 85% of tumors. Chromosome 1p aberrations both as structural and numerical abnormalities were found to be one of the most frequently occurring aberrations among T-cell neoplasias. The most frequent 1p breakpoints involve band 1p32-36 [10]. Interestingly, both deletions and translocations frequently observed in T-ALL involve this same chromosomal region [11], the most common genetic change observed being a 90 Kb deletion resulting in fusion of the SIL promoter to the *TAL-1* gene [8, 12-15]. *TAL1* 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). *TAL1* contains seven acceptor deletion sites (taldb1 to taldb7), with two being involved in almost all cases (taldb1 and taldb2). The vast majority (~90%) of *TAL1* deletions is located between Sildb1 and taldb1, and is known as *TAL1* deletion type I. The *TAL1* deletion type-2 occurs between Sildb1 and taldb2 [15]. Disruption of the *TAL1* locus on chromosome 1p32 represents one of the most commonly recognized genetic abnormalities in T-ALL. *TAL1* codes for a basic helix-loop-helix (bHLH) transcription factor [7, 16] which is normally expressed by haemopoietic precursors, by the megakaryocyte, mastocyte and erythroid lineages and by endothelial cells [17] but not by normal T cells. The *TAL1* gene is disrupted by at least two mechanisms: translocation and site-specific deletion [16]. The t(1;14) (p32;q11), involving *TAL1* and the *TCR*d locus on chromosome 14q11, occurs in 3% of childhood T-ALL [7, 9, 12] and rare variant translocations [9] have been identified. A more frequent,

approximately 90 kbp, site-specific deletion upstream to *TAL1*, *tald*, leads to replacement of the 50 part of *TAL1* by regulatory sequences from the *SIL* gene. It occurs in 6–26% of T-ALL, preferentially in cases which express TCR ab [18-19]. At least five *TAL1* deletion breakpoints have been reported [19], with *taldb1* and, to a lesser extent, *taldb2* occurring most frequently. Detection of *tald* is most frequently undertaken by PCR from DNA, with screening limited to detection of *tald1* and *tald2*. Several primer pairs or Southern blot analysis with *SIL* and 50 *TAL1* probes are necessary to detect all variant breakpoints [20].

However, to the best of our knowledge, the role of *TAL1* genetic polymorphisms in the susceptibility, clinical features and biological characteristics of T-ALL and T-NHL remain unknown at least in Libya. Therefore, this study aims, for the first time, to explore the possible association between the *TAL1* genetic variations (exons 2 and 3) and T-ALL and T-NHL risk using DNA-PCR and direct DNA sequencing. Also, clarifying the effect of *TAL1* mutations on clinical parameters is of particular interest in our study.

Materials and Methods

Patient Population

The case–control study consisted of 15 controls, 20 T-ALL patients and 10 T-NHL patients. Unrelated healthy controls were recruited from general population. T-ALL and T-NHL patients attending to Department of Oncology, Tripoli Medical Cancer, at Tripoli, Libya were included in the present study. For sample preservation and genetic analysis, local institutional review board approval and informed consent from all participants were obtained by the Ethics Committee of the First Affiliated Tripoli Medical Cancer at Tripoli, Libya. T-ALL and T-NHL cancer was confirmed by histological examination after hysterectomy or myomectomy. The median age of the case series was 23 years (range, 3 - 66 years), and all of studied groups were males (60.5%), and all were Libyans (undefined ethnic group).

Sampling and DNA Extraction

Blood samples (five-mL) were taken from all participants by peripheral antecubital venous puncture, drawn into sodium EDTA tube and were then stored at −20°C until analysis. Genomic DNA was extracted from the blood samples of controls, T-ALL patients, and T-NHL patients by a standard procedure (standard phenol and chloroform) [20]. The integrity of extracted genomic DNA and its concentration were measured by UV-spectrophotometry (BioPhotometer, eppendorf), with absorbance of A260/A280 nm ratios at pH 8.0 between 1.7 and 2

for all samples, and ethidium-bromide fluorescence of DNA separated by 1.5% AGE (agarose gel electrophoresis). The quality was checked by amplifying β-actin housekeeping gene. Purified DNA was stored at -70 °C till being used.

Molecular Characterization of T-ALL and T-NHL Patient Samples

TAL1 mutations were analyzed by PCR amplification of *TAL1* exons 2 and 3, and followed by direct bidirectional DNA sequencing using previously published primers and PCR conditions [5, 21].

PCR amplification was carried out using a Perkin-Elmer 480 thermocycler (Applied Biosystems) in a 25 µl reaction mixture containing 10X PCR buffer, 1.5 mM MgCl , 200 µM of dNTP's (AB Gene), 1 U of Hotstart Taq Polymerase (AB Gene), 10 pmol of forward and reverse primers with 100 ng of genomic DNA (Table 1). The reaction was heated at 95°C for 5 min, then amplified for 30 cycles [95°C /30 sec, annealing/30 sec (57°C for exon 2 and 50°C for exon 3) and 72°C/30 sec] followed by 5 min final extension at 72°C. PCR amplification and the absence of primer dimers were confirmed by analysis of cycling and melting curves. Negative controls (water instead of DNA) were included in all PCR experiments. Linearity and specificity of PCR amplification were validated before quantification. *TAL1* exons 2 and 3 amplified products (198 bp and 235 bp, respectively) were visualized by electrophoresing on a 1.5% AGE. All PCR amplifications resulted in a single and specific product of the expected size (Figure 1). The banding pattern of extracted genomic DNA did not show any evidence of DNA degradation (Figure 1).

Qualified amplicons (PCR products) were cleaned using exonuclease I and shrimp alkaline phosphatase (SAP). Sequencing reaction was performed using Big Dye Terminator Cycle Sequencing Ready Reaction kit v3.0 (Applied Biosystems) at 5′→3′ direction, as indicated by the manufacturer. The nucleotide sequence detection was performed in the ABI Prism 310 Genetic Analyzer (Applied Biosystems) using standard protocols. Finally, the sequencing results were compared with the standard sequence of *TAL1* gene (exons 2 and 3).

Statistics

All calculations were performed using the SPSS software package (version 20.0). Data were expressed as mean ± SEM. The patients' characteristics were analyzed by the chi-squared (χ^2) or Fisher's exact tests for univariate analysis. Potential confounders, such as age, sex, and biochemical measurements were also studied. Differences between controls and cases were compared by either Student's t-test or one way ANOVA, as appropriate. *P* values less than 0.05 were deemed statistically significant.

Table 1. Primers used to amplify exons 2 and 3 of the *TAL1* gene.

Exon	Primer sequence	Amplicon size (bp)	Annealing temperature
2	FP: 5'GGATTGTGAGAGTGCGTTCA3' RP: 5'TCAGATCCCTGCTGAGAACA3'	198	57 °C
3	FP: 5'ATGTATTCGGGAGCCAGTTG3' RP: 5'ACCCACGAAACTGACCAGAA3'	235	50 °C

FP: forward primer; RP: reverse primer; bp: base pair; *TAL1* gene: T-Cell Acute Lymphocytic Leukemia 1 gene.

Reproducibility of the results was confirmed by randomly repeating the mutation analysis for 5% of all DNA samples. The sequencing was carried out in the Biochemistry Department, Hospital of Infants, Tunisia.

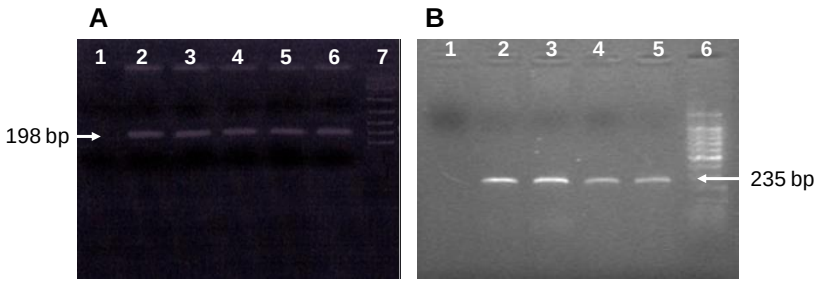

Figure 1. Gel electrophoresis of *TAL1* exons 2 and 3 PCR products using allele-specific primers. (**A**) Lane 1 represents the negative control; lanes 2 to 6 show amplified products of *TAL1* exon 2 (198 bp); lane 7 correspond to the 500 bp DNA marker. (**B**) Lane 1 corresponds to the negative control; lanes 2 to 5 show amplified products of *TAL1* exon 3 (235 bp); lane 6 corresponds to the molecular size marker 500 bp. *TAL1* gene: T-Cell Acute Lymphocytic Leukemia 1 gene; PCR: polymerase chain reaction; bp: base pair.

Results

TAL1 Gene Mutations and T-ALL Patient Samples

TAL1 exon 2 mutations were identified in 7.7% adult and 12.5% adolescent T-ALL patients analyzed. Overall, nine *TAL1* mutations were documented in this study, involving exon 2. Among these, frame-shift and missense mutations accounted for 44.4% (4/9) and 55.6% (5/9), respectively, of all *TAL1* exon 2 mutations. These mutations have not been described previously. The frequency of

specific *TAL1* mutations identified in the present study is listed in Table 2 and graphically depicted in Figure 2. Furthermore, in our study sequence *TAL1* exon 3 revealed no nucleotide variation for the T-ALL cancer patients analyzed, suggesting that *TAL1* exon 3 is not, to date, the main known genetic risk factor in T-ALL at least in this study.

Table 2. Characteristics of T-All patients with a *TAL1* gene mutation.

Case n.	Sex	Age	Mutation(s)	Exon	Mutation Type	Protein level	Status
1 (ALL)	M	15	1022insC	2	Frame-shift	Val340fsX	N
			1024T>A		Missense	Val341Asp	N
			1060T>G		Missense	Val353Gly	N
			1066delC		Frame-shift	Ala355fsX	N
			1144T>G		Missense	Val381Gly	N
2 (ALL)	F	35	991delA	2	Frame-shift	Asp331fsX	N
			1036delC		Frame-shift	Thr345fsX	N
			1086C>A		Missense	His360Gln	N
			1087A>T		Missense	Thr361Ser	N

M: male; F: female; T-ALL: T-cell acute lymphoblastic leukaemia; *TAL1* gene: T-cell acute leukemia 1 gene; ins: insertion; del: deletion; R: reported; N: novel; X: Stop codon; fs: frame shift.

TAL1 Mutations and T-NHL Patient Samples

TAL1 exon 2 mutations were detected in 16.7% of the adult T-NHL patients analyzed. Overall, 5 *TAL1* mutations were documented in this study, involving exons 2. Among these, missense and frame-shift mutations accounted for, respectively, 60% (3/5) and 40% (4/5) of all *TAL1* exon 2 mutations. The frequency of specific *TAL1* exon 2 mutations identified in the present study listed in Table 3 and graphically depicted in Figure 3. Sequence analysis of *TAL1* exon 3 revealed no nucleotide changes for the T-NHL cancer patients analyzed.

Taken together, our results illustrate that a variation in *TAL1* exon 2, but not exon 3, gene play a role in the T-ALL and T-NHL in the studied population.

Association of *TAL1* Gene Mutations with T-All and T-NHL Patient Clinical Features

The clinical features of *TAL1* wild type and *TAL1* mutant T-All and T-NHL cases are compared in Table 4. T-All and T-NHL cases with *TAL1* exon 2 positive mutations had a higher PLT counts (P=0.177 and P=0.165, respectively). T-All cases, but not T-NHL cases, with *TAL1* exon 2 positive mutations had a lower WBC than the cases with *TAL1* exon 2 negative mutations counts (Table 4). The average age of the T-All cases with *TAL1* exon 2 mutations was higher than the average age of the cases without *TAL1* exon 2 mutations, but they have not

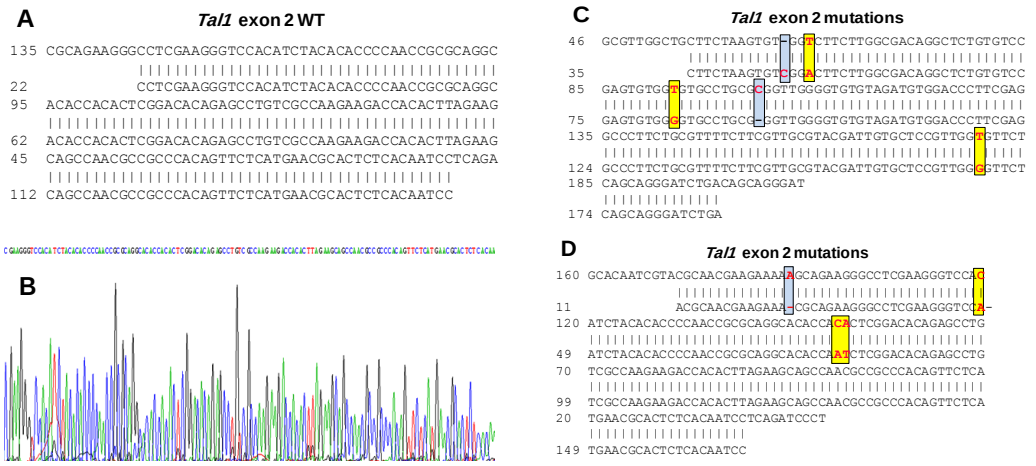

Figure 2. *TAL1* gene mutations in T-ALL patients. (A-C) Representative DNA sequencing chromatograms of T-ALL genomic DNA samples showing mutations in exon 2 of *TAL1*. WT: wild type. T-ALL: T-cell acute lymphoblastic leukaemia; *TAL1* gene: T-cell acute lymphoblastic leukemia 1 gene.

reached statistical significance (P=0.588). In contrast, the average age of the T-NHL cases with *TAL1* exon 2 mutations was lower than the average age of the cases without *TAL1* exon 2 mutations (P=0.576).

Discussion

T-cell acute lymphoblastic leukemia (T-ALL) and lymphoblastic T-cell non-Hodgkin's lymphoma (T-NHL) are closely associated disorders, and distinguishing between the two is difficult [1-2, 22-24]. *TAL1* is amongst the most frequently deregulated oncogenes [22]. In physiological conditions, *TAL1* is a regulatory gene that promotes access to alternative fates in hematopoiesis. *TAL1* gene alterations and T-ALL and T-NHL susceptibility has been investigated in many studies [22-25], but the reported results are not always consistent. Thus, the present study was designed to identify the prevalence of *TAL1* gene (exons 2 and 3) alterations in T-ALL and T-NHL Libyan patients.

TAL1 gene (exons 2) mutations accounts for 15% of pediatric and 25% of adult T-ALL cases [26]. Importantly, significant differences in outcome are present between paediatric and adult T-ALL [26]. In spite of > 70% of children achieve long lasting complete remissions, only 50% of adult T-ALL patients are currently

cured. In addition, pediatric and adult T-ALLs exhibit marked differences in the frequency of specific genetic lesions [27-29]. For example, chromosomal translocation and aberrant expression of the *TAL1* and *TLX3* oncogenes are highly prevalent in children, but rare in adults. In contrast, translocations activating *TLX1*

Table 3. Characteristics of T-NHL patients with a *TAL1* gene mutation.

Case n.	Sex	Age	Mutation(s)	Exon	Mutation Type	Protein level	Status
1 (NHL)	M	3	1098delAG	2	Frame-shift	Arg366fsX	N
			1056delG		Frame-shift	Gly352fsX	N
			1014insT		Missense	Lys338Lys	N
2 (NHL)	F	18	1099G>C	2	Missense	Arg366Thr	N
			1101G>A		Missense	Gly346Asp	N

M: male; F: female; T-NHL: T-cell non-Hodgkin's lymphoma; *TAL1* gene: T-cell acute leukemia 1 gene; ins: insertion; del: deletion; R: reported; N: novel; X: Stop codon; fs: frame shift.

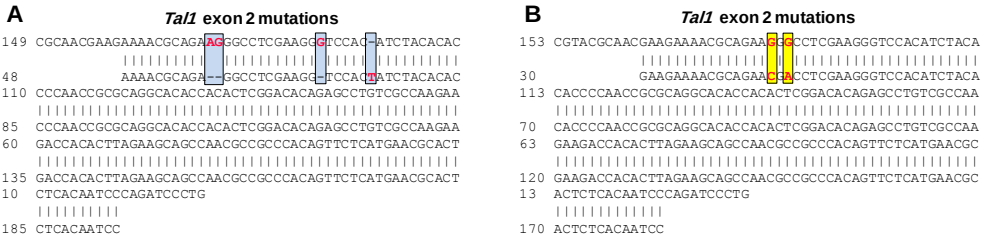

Figure 3. *TAL1* gene mutations in T-NHL patients. (A-B) Representative DNA sequencing chromatograms of T-NHL genomic DNA samples showing mutations in exon 2 of *TAL1*. WT: wild type. T-NHL: T-cell non-Hodgkin's lymphoma; *TAL1* gene: T-cell acute leukemia 1 gene.

Table 4. T-All and T-NHL Patients' characteristics and *TAL1* exon 2 mutations.

Patients' characteristics	*TAL1* wild type	*TAL1* mutated	*P*
T-All			
PLT, ×109/L(range)	166.41 ± 31.82	326.5 ± 176.5	0.177
WBC count, 10^9/L (range)	11.59 ± 4.36	8.0 ± 0.001	0.810
Age *(years)*	20.77 ± 2.17	25.0 ± 10.0	0.588
T-NH			
PLT, ×109/L(range)	178.83 ± 29.91	304 ± 0.01	0.165
WBC count, 10^9/L (range)	4.58 ± 0.98	6.5 ± 3.50	0.493
Age *(years)*	32.5 ± 5.44	24.50 ± 6.5	0.576

PLT: platelets; WBC: white blood cell; T-ALL: T-cell acute lymphoblastic leukaemia; T-NHL: T-cell non-Hodgkin's lymphoma; *TAL1* gene: T-cell acute leukemia 1 gene; P value: *rank sum test; #Fisher's test.

are rarely identified in pediatric leukemias but represent one third of adult T-ALL cases [29]. Almost 25% of patients with T-ALL have tumor-specific rearrangements of the *TAL1* gene [27-28]. Although *TAL1* expression has not been observed in normal lymphocytes, *TAL1* gene products are readily identified in leukemic cells that harbor a rearranged *TAL1* allele. Hence, it has been suggested that ectopic expression of *TAL1* promotes the T-ALL development. In our study, *TAL1* exon 2 mutations were identified in 10% (n=20) of T-ALL patients analyzed. In contrast to previous findings [5], we have observed no male excess of *TAL1* mutations in T-ALL patients. We detected excess of adolescent (12.5%, n=8) over adult (7.7%, n=13) cases of *TAL1* exon 2 mutations in T-ALL patients. In contrast with our study, the *TAL1* mutations were found more frequent in adult T-ALL [5]. Overall, *TAL1* mutations were reported in this study, implicating exons 2. Among these, frame-shift and missense mutations accounted for, respectively, 44.4% and 55.6% of all *TAL1* exon 2 mutations (Table 2, Figure 2). These mutations have not been reported previously.

In fact, T-NHL is usually differentiated from T-ALL by minimal or absent bone marrow and peripheral blood involvement, normal WBC count, normal hemoglobin levels and lack of organomegaly. Because this distinction does not apply in every case, a presence of less than 25% of blasts in the bone marrow is currently used to define T-NHL from T-ALL [4]. The relationship between *TAL1* variants and T-NHL cancer risk has been investigated in several studies [4, 15, 24]. In our study, *TAL1* exons 2 mutations were detected in 20% T-NHL patients analyzed. Again in contrast to a previous study [5], we found no difference in the rates of *TAL1* mutated cases between male and female T-NHL patients from Libya. Missense and frame-shift mutations accounted for, respectively, 60% and 40% all *TAL1* exon 2 mutations. In the present study, sequence of *TAL1* exon 3 showed no sequence variation for the cancer patients analyzed. The absence of *TAL1* exon 3 mutations in this study would have occurred due to the number of patient samples used.

Comparing the clinical features of cases showing *TAL1* mutation positive and *TAL1* mutation negative revealed that WBC counts the only significant variable between the two groups [5, 30]. Previously, it has been shown that the expression of T-cell markers CD3, CD4, and CD7 were less in cases showing *TAL1* mutations compared to *TAL1* mutations negative cases but they have not reached statistical significance. Moreover, it has been shown that the patients with *TAL1* rearrangements at presentation are usually associated with high WBC count [5], CNS disease, immunophenotype of CD2+ and CD5+ [30]. In our study, we also found that *TAL1* positive mutations were associated with higher PLT counts and WBC counts in cancer patients analyzed. Our study suggests that *TAL1* mutations may cooperate with these genetic abnormalities during T-cell leukemogenesis.

Collectively, this study did not show any evidence of a significant correlation between *TAL1* variants and increased risk of T-ALL and T-NHL. In spite of study is the first study to explore T-ALL and T-NHL in Libya at molecular level, the large sample size offers more reliable conclusions regarding T-ALL and T-NHL among Libyans. In our study, we found no difference in the rates of *TAL1* mutated cases between male and female T-NHL patients from Libya. Together with previous investigations, our findings call for closer examination of entire coding region of *TAL1* gene in T-ALL and T-NHL patients of both sexes in ethnically defined populations. Also, the present study makes it necessary to study other molecular abnormalities in T-ALL and T-NHL such as *JAK1* mutation, *p16 INK4a* deletion, *PHF6* mutation, *Notch1* mutation, and *TLX1* (HOX11) gene expression to explore whether they contribute to the high prevalence of T-ALL and T-NHL in Libyan patients with T-ALL and T-NHL.

Authors' Contributions

Amal E. Elarifi, Othman A. El-Ansari and Mohamed A. Al-Griw substantially contributed to the conception and design of the study, acquisition, analysis and interpretation of data; all authors drafted the article and made critical revisions related to the intellectual content of the manuscript, and approved the final version of the article to be published.

Acknowledgements

The authors wish to thank all academic staff who gave us scientific advice and technical support in the Department of Zoology, Faculty of Science, University of Tripoli, Tripoli, Libya. The authors extend their thanks to Biochemistry Laboratory staff at the Tunisian Infant Hospital for their assistance.

References

[1] Reinherz, E.L., Nadler, M., Sallin, S.E., Schlossman, S.F. (1979). Subset derivation of T-cell acute lymphoblastic leukemia in man. Journal of Clinical Invest, **64**: p. 392-397.

[2] Roper, M., Crist, W.M., Metzgar, R. (1983). Monoclonal antibody characterization of surface antigens in childhood T-cell lymphoid malignancies. Blood, **61**: p. 830-837.

[3] Crist, W.M., Shuster, J.J., Falleta, J. (1988). Clinical features and outcome in childhood T-cell leukemia-lymphoma according to stage of thymocyte differentiation: a Pediatric Oncology Group study. Blood, **72**: p. 1891-1897.

[4] Bernard, A., Boumsell, L., Patte, C., Lemerle, J. (1986). Leukemia versus lymphoma in children. A worthless question?. Med Pediatr Oncology, **14**: p. 148-157.

[5] Sudhakar, N., Nirmala, K., Rajalekshmy, K.R., Rajkumar, T. (2008). Does TAL-1 Deletion Contribute to the High Incidence of T-Cell Acute Lymphoblastic Leukemia in South Indian Patients?. Asian Pacific Journal of Cancer Prevention, **9**: p. 127-130.
[6] Rajalekshmy, K.R., Abitha, A.R., Pramila, R. (1997). Immunophenotypic analysis of T-cell acute lymphoblastic leukemia in Madras, India. Leukemia Research, **21**: p. 119-124.
[7] Begley, C.G., Aplan, P.D., Davey, M.P. (1989). Chromosomal translocation in a human leukemic stem-cell line disrupts the T-cell antigen receptor d-chain diversity region and results in a previously unreported fusion transcript. Proc Natl Acad Sci USA, **86**: p. 2031.
[8] Brown, L., Cheng, J-T., Chen Q. (1990). Site specific recombination of the TAL-1 gene is a common occurrence in human T cell leukemia. EMBO Journal, **9**: p. 3343-3351.
[9] Aplan, P.D., Lombardi, D.P., Reaman, G.H. (1992). Involvement of the putative Hematopoietic Transcription factor SCL in T-Cell Acute Lymphoblastic Leukemia. Blood, **79**: p. 1327.
[10] Offit, K., Jhanwar, S.C., Ladanyi, M., Filippa, D.A., Chaganti, R.S.K. (1991). Cytogenetic analysis of 434 consecutively ascertained specimens of non-Hodgkin's lymphoma: correlations between recurrent aberrations, histology, and exposure to cytotoxic treatment. Genes Chrom Cancer, **3**: p. 189-201.
[11] Raimondi, S.C. (1993). Current status of cytogenetic research in childhood acute lymphoblastic leukemia. Blood, **81**: p. 2237-2251.
[12] Bernard, O., Guglielmi, P., Jonveaux, P. (1990). Two distinct mechanisms for the SCL gene activation in the t(1;14) translocation of T-cell leukemias. Genes Chromos Cancer, **1**: p. 194-208.
[13] Aplan, P.D., Lombardi, D.P., Reaman, G.H., Sather, H.N., Hammond, G.D., Kirsch, I.R. (1992). Involvement of the putative hematopoietic transcription factor SCL in T-cell acute lymphoblastic leukemia. Blood, **79**: p. 1327-1333.
[14] Bash, R.O., Crist, W.M., Shuster, J.J. (1993). Clinical features and outcome of T-cell acute lymphoblastic leukemia in childhood with respect to alterations at the TAL1 locus: a Pediatric Oncology Group study. Blood, **81**: p. 2110-2117.
[15] Breit, T.M., Mol, E.J., Wolvers-Tettero, I.L., Ludwig, W-D., van Wering, E.R., van Dongen, J.J.M., Site-specific deletions involving the TAL1 and sil genes are restricted to cells of the T cell receptor a/b lineage: T cell receptor d gene deletion mechanism affects multiple genes. Journal of Exp Med, 1993, **177**: p. 965-977.
[16] Baer, R. (1993). TAL1, TAL2 and LYL1: a family of basic helix-loophelix proteins implicated in T-cell acute leukaemia. Seminars in Cancer Biology, **4**: p. 341-347.
[17] Kallianpur, A.R., Jordan, J.E., Brandt, S.J. (1994). The SCL/Tal-1 gene is expressed in progenitors of both the hematopoietic and vascular systems during embryogenesis. Blood, **83**: p. 1200-1208.
[18] Aplan, P.D., Lombardi, D.P., Ginsberg, A.M., Cossman, J., Bertness, V.L. & Kirsch, I. (1990). Disruption of the human SCL locus by 'illegitimate' V-(D)-J recombinase activity. Science, **250**: p. 1426-1429.

[19] Bernard, O., Lecointe, N., Jonveaux, P., Souyri, M., Mauchauffe´, M., Berger, R., Larsen, C.-J. & Mathieu-Mahul, D. (1991). Two sitespecific deletions and t(1;14) translocation restricted to human Tcell acute leukemias disrupt the 50 part of the TAL1 gene. Oncogene, **6**: p. 1477-1488.

[20] Huang, W., Kuang, S.Q., Huang, Q.H., Dong, S., Zhang, T., Gu, L.J., Ching, L.M., Chen, S.J., Chang, L.C., Chen, Z. (1995). RT/PCR detection of SIL-TAL1 fusion mRNA in chinese T-cell acute lymphoblastic leukemia (T-ALL). Cancer Genetics and Cytogenetics, **81**: p. 76-82.

[21] Sanger, F., Nickler, S., Coulson, A.R. (1977). DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA, **74**: p. 5463 -5467.

[22] Navarro, J.M., Touzart, A., Pradel, L.C., Loosveld, M., Koubi, M., Fenouil, R., Le-Noir, S., Maqbool, M.A., Morgado, E., Gregoire, C., Jaeger, S., Mamessier, E., Pignon, C., Hacein-Bey-Abina, S., Malissen, B., Gut, M., Gut, I.G., Dombret, H., Macintyre, E.A., Howe, S.J., Gaspar, H.B., Thrasher, A.J., Ifrah, N., Payet-Bornet, D., Duprez, E., Andrau, J.C., Asnafi, V., Nadel, B. (2015). Site- and allele-specific polycomb dysregulation in T-cell leukaemia. Nature Communication, **23**: p. 6094.

[23] Kikuchi, A., Hayashi, Y., Kobayashi, S., Hanada, R., Moriwaki, K., Yamamoto, K., Fujimoto, J., Kaneko, Y., Yamamori, S. (1993). Clinical significance of TAL1 gene alteration in childhood T-cell acute lymphoblastic leukemia and lymphoma. Leukemia, **7**: p. 933-938.

[24] Perotti, D., Pettenella, F., Luksch, R., Giardini, R., Gambirasio, F., Ferrari, D., Fossati-Bellani, F., Biondi, A. (1999). Molecular analysis of 1p32 genetic involvement in pediatric T-cell non-Hodgkin's lymphoma. Haematologica, **84**: p. 110-113.

[25] Kikuchi, A., Kobayashi, S., Hanada, R., Moriwaki, K., Yamamoto, K., Fujimoto, J., Kaneko, Y., Yamamori, S., Miwa, H., Kita, K., Hayashi, Y. (1998). TAL1 gene analysis in T-cell malignancies. Rinsho Ketsueki, **39**: p. 259-266.

[26] Pui, C.H., Robison, L.L., Look, A.T. (2008). Acute lymphoblastic leukaemia. Lancet, **371**: p. 1030-1043.

[27] Zhou, Y., Kurukuti,S., Saffrey, P., Vukovic, M., Michie, A.M., Strogantsev, R., West, A.G., Vetrie, D. (2013). Chromatin looping defines expression of TAL1, its flanking genes, and regulation in T-ALL. Hematopoisis and Stem Cells, 19;122 (26):4199-209.

[28] Bash, R.O., Hall, S., Timmons, C.F., Crist, W.M.Amylon, M.M., Smith, R.G., Baer, R. (1995). Does activation of the TAL1 gene occur in a majority of patients with T-cell acute lymphoblastic leukemia?. A pediatric oncology group study, **86**: p. 666-676.

[29] Ferrando, A.A., Neuberg, D.S., Dodge, R.K., Paietta, E., Larson, R.A., Wiernik, P.H., Rowe, J.M., Caligiuri, M.A., Bloomfield, C.D., Look, A.T. (2004). Prognostic importance of TLX1 (HOX11) oncogene expression in adults with T-cell acute lymphoblastic leukaemia. Lancet, **363**: p. 535-536.

[30] Aplan, P.D., Lombardi, D.P., Reaman, G.H. (1992). Involvement of the putative Hematopoietic Transcription factor SCL in T-Cell Acute Lymphoblastic Leukemia. Blood, **79**: p. 1327.