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HOX11L2 Expression in Egyptian Pediatric T-Cell Acute Lymphoblastic Leukemia

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ABSTRACT

Background and Purpose: A cryptic translocation t(5;14) (q35;q32) brings HOX11L2 on chromosome 5 under the influence of CTIP2 on chromosome 14 which is highly expressed during T lymphoid differentiation. Studies indicate that t(5;14) and/or HOX11L2 ectopic expression is restricted to T-lineage ALL and is more frequent in children (about 20-25%) than in adults and can represent a frequent specific genetic alteration in childhood T ALL. The deregulation of HOX11 with or without the presence of t(10;14) (q24;q11) or t(7;10) (q35;q24) has been described in 4% to 10% of children with T-ALL respectively and lead to HOX11 gene activation. The aim of this work is to study the frequency of HOX11L2 and HOX11 expression in Egyptian pediatric T-ALL cases and its clinical relevance.

Patients and Methods: Sixty five pediatric patients with newly diagnosed T-ALL, who presented to the National Cancer Institute, Cairo-Egypt, during the period between 1st of January 2004 and end of February 2005, were immunophenotyped by flowcytometry and tested by molecular analysis (RT-PCR) for HOX11L2 expression. Cases positive for HOX11L2 expression were retested whenever bone marrow was done during follow-up. HOX11 expression was tested in 58 cases. As controls, 20 children with B-lineage ALL and 20 with AML were also examined.

Results: No expression of HOX11L2 was detected in B-ALL or AML samples. T-ALL cases were mostly compartment II [32/65, (49.2%)], 26.2% in compartment I and 24.6% in compartment III. Out of the 65 T-ALL cases, 8 (12.3%) expressed HOX11L2 (3 in compartment I, 2 in compartment II and 3 in compartment III). Positive cases had a median age of 10.5 years and 5/8 cases (62.5%) had total leukocytic counts >100x10^9/l. During follow-up, positive cases in complete remission showed no HOX11L2 expression while the relapsing case showed HOX11L2 expression 2 months before hematological relapse. The expression of HOX11 was observed in 2 of 58 (3.4%) T-ALL samples tested. None of the HOX11L2 positive cases tested (7/8) expressed HOX11.

Conclusion: Our T-ALL cases showed a lower incidence of HOX11L2 expression than recorded in western countries but HOX11 expression is comparable. HOX11L2 expression may be used as a marker for minimal residual disease detection, however, further study on a larger scale is recommended.

Key Words: *T ALL - HOX11 - HOX11L2.*

INTRODUCTION

Acute leukaemias are the most common form of cancer in children, accounting for approximately one-third (30%) of all juvenile neoplasms (age <16 years). The majority of these cases are classified as acute lymphoblastic leukaemia (ALL) with about 15% and 26% of these being of T-cell phenotype (T-ALL) in developed countries and Egypt respectively **[1,2]**. Despite continual improvements in treatment over many years, T ALL is still associated with a significant mortality, and relapsed ALL continues to contribute greatly to the overall morbidity and mortality of childhood cancer **[3-6]**. About 50% of T ALL has recognizable genetic abnormalities. Several of these abnormalities are specifically associated with T-ALL. The Genetic abnormalities can be divided into: deletions and translocations. The more common genetic defects in T-ALL are submicroscopic deletions of p16/INK4 in 40% to 80% **[7,8]** or SIL-TAL1 in 10% to 25% **[9-11]**. Most famous translocations are t(11;14), t(10;14) and t(1;14) **[12]**.

The most common recurring breakpoints are within the 14q11, 7q32-q36 and 7p15 bands, which contain the T-cell receptor (TCR) genes TCRα/δ, TCRβ and TCRγ, respectively **[3-6]**.

Molecular analysis of the chromosomal breakpoints has identified several T-cell oncogenes; most of them have been formally shown

to be tumorigenic [13]. Remarkably, the majority of T-cell oncogenes belong to a number of classic transcription factor families whose expression is most often intended for lineages other than T cells. The factors deregulated in T-ALL comprise the HOX11 homeobox genes, LMO1 and LMO2 which contain duplicated LIM zinc-finger motifs, and MYC, TAL1 (SCL), TAL2 and LYL1 which all encode helix-loop-helix proteins. Studies on gene expression profiles in T-ALL confirmed the activation of these transcription factors to be a hallmark in these leukaemias [14].

Recognized T-ALL oncogenic pathways include transcriptional deregulation by juxtapositioning to one of the TCR loci, resulting in transcriptional deregulation of genes such as HOX11/TLX1, LMO2, LMO1, LYL1, and TAL1/SCL, each of which is present in less than 10% of cases [12].

The HOX11 and the closely related HOX11L2 genes are called orphan homeobox genes because they are located outside the 4 mammalian HOX clusters. They were both identified at recurrent chromosomal breakpoints in T-ALL [15,16].

HOX11 is not expressed in healthy T cells [17]. Translocation t(10;14) is a nonrandom alteration observed in both T-ALL and T-cell lymphoblastic lymphomas [18]. It leads to high HOX11 expression. There is some evidence that HOX11 may play an important role in leukemogenesis [17]. The deregulation of HOX11 in the t(10;14) (q24;q11) or t(7;10) (q35;q24) has been described in 4% to 10% of children with T-ALL respectively and lead to HOX11 gene activation by bringing HOX11 coding sequence under the transcriptional control of regulatory sequence of the TCR gene [6,17,19]. HOX11 over expression has been demonstrated in absence of 10q24 rearrangement. HOX11 expression in leukaemic blasts conferred a prognostic advantage [1,12].

Cryptic translocations were also recognized by fluorescence insitu hybridization (FISH), with the most common being the t(5;14) (q35;q32), leading to over expression of HOX11L2/TLX3 in 25% to 30% of pediatric T-ALL [14-17,20,21]. This translocation leads to the ectopic expression of HOX11L2 possibly by bringing it under the influence of regulatory elements of CTIP2, a gene highly expressed during T-lymphoid differentiation. It seems to be restricted to T-lineage ALL and is more frequent in children than in adults [17]. Another rare translocation observed in T-ALL, t(5;14) (q33;q11), involves the T-cell receptor (TCRα/δ) gene locus on chromosome 14. Its breakpoint on chromosome 5 is located 2kb upstream of the HOX11L2 coding sequence [22]. In this respect it could lead to HOX11L2 transcription deregulation [17].

The aim of this study was to prospectively evaluate the frequency and the clinical relevance (possible role in follow-up and the prognostic value) of HOX11 and HOX11L2 expression in childhood T-ALL.

PATIENTS AND METHODS

Patients:

Patients were children with T-ALL presenting to the pediatric oncology department at NCI, Cairo University, from beginning of January 2004 to end of February 2005. Sixty five patients were diagnosed as T-ALL during that time, included 48 males and 17 females with age ranging from 0.5-17 years with a median of 10 years. Bone marrow (BM) or peripheral blood (PB) samples were obtained at the time of diagnosis and during cytologic remission or at relapse whenever possible. Diagnosis was based on standard morphologic, cytochemical parameters of leukemic cells and on the expression of T-cell antigens and the absence of B-cell and myeloid antigens. Clinical data of patients are summarized in Table (1). Samples from children with B-ALL (20 patients), and acute myeloblastic leukemia (AML, 20 patients) diagnosed during the same period of time were also tested.

Written informed consent was obtained from the patients' parents and the protocol was approved by the Institution Research Board.

Immunophenotyping:

Immunophenotypic analysis was performed on peripheral blood or bone marrow samples taken at the time of diagnosis. It was assessed by multicolor flow cytometry (Coulter Epics XL, Hialeh). A wide panel of FITC (fluorescin) or PE (phycoerythrin) conjugated monoclonal antibodies (MoAbs) was used. The panel used is listed in Table (2). Double and Triple marker

labeling was performed, including proper isotype controls. All MoAbs and isotypic controls were supplied from Beckman Coulter and Dako Cytomation (Denmark).

Detection of surface markers by direct staining:

The whole blood staining method was performed. Ten µl labeled Mo Ab was added to 100µl whole blood (or BM), incubated in the dark for 20 minutes, hemolyzed (Optilyse® No-wash Lysing solutions, A11894, Beckman Coulter) and washed by PBS then analyzed.

Detection of intracellular markers (CD3 and CD22):

One-hundred µl of whole blood was lysed using lysis solution (Becton & Dicknson) for 10 minutes. Cells were washed once and resuspended in 1ml PBS. A mixture of 500µl 4% paraformaldehyde as fixative, 500µl PBS and 5µl tween 20 as detergent was added to the cells and incubated for 10min. The cells were washed and 10µl MoAb was added and incubated for 30min at 4°C. Cells were washed, suspended in 500µl PBS and analyzed [23].

Any antigen was considered positive when 20% of blast cells were stained above the negative control except for CD34 where 10% was considered positive.

RNA methods:

Total RNA was extracted from patient samples using Total RNA isolation kit, Purescript, Gentra Minneapolis USA according to the manufacturer's instructions. Whole blood and/or BM was collected on EDTA and processed immediately. The red blood cells (RBCs) in 300ul blood/BM were lysed by RBCs lysis solution, then the cell pellet lysed by cell lysis solution. The protein and DNA were precipitated by protein-DNA precipitation solution. The supernatant containing RNA was collected on 100% isopropanol and centrifuged to precipitate RNA which was then washed by ethanol. RNA pellet was left to dry then dissolved in 20ul RNA hydration solution. RNA was stored at -80ºC until used.

RNA was reverse transcribed from 1µg total RNA in a final volume of 20µL containing reverse transcription-polymerase chain reaction (RT-PCR) buffer (1mM each dNTP, 3mM $MgCl_2$, 75mM KCl, 50mM Tris-HCl pH 8.3), 10U RNAsin (Promega, Madison, WI), 100mM dithiothreitol, 100U Superscript II (Gibco-BRL, Cergy Pontoise, France), and 25µM random hexamers. One hundred nanograms cDNA equivalent of RNA was analyzed in each PCR experiment. PCR was carried out in a final volume of 50µL with 0.5U AmpliGold polymerase (PE Applied Biosystems, Foster City, CA), 200µM of each dATP, dCTP, dGTP and dUTP, 25pmol each primer and 2.5mM $MgCl_2$. Cycle parameters were set for 10 minutes at 95°C and for 15 seconds at 95°C, 40 seconds at 60°C and 40 seconds at 72°C for 35 cycles. PCR products were run on 2% agarose gel at 120 Volt for 20 minutes. Cases were considered positive when a single 244-nucleotide fragment was observed.

Primers [16]: Screening for HOX11L2 and HOX11 expression was carried out by Standard RT-PCR using the following primers:

- HOX11L2 2Fo: GCGCATCGGCCAC-CCCTACCAGA;
- HOX11L2 3Rw: CCGCTCCGCCTC-CCGCTCCTC;
- HOX11-712Fo: CTGGCCAAGGCGCT-CAAAATG; and
- HOX11-810Rw: GGCCTCCCGTTCCTCCG-CAGTC.

Positive control for HOX11L2:

T ALL cases with 100% blasts in peripheral blood were tested for HOX11L2, 2 cases were positive and showed the single 244-nucleotide fragment. They were relapsed cases and hence were not included in the study and either was used as a positive control for every run.

Treatment:

Patients were treated according to the Egyptian NCI treatment protocol modified from the total therapy study XIII-B of St. Jude Children's Research Hospital (SJCRH) [24]. It included 6 weeks of induction, 2 weeks of consolidation, and 120 weeks of continuation therapy.

Induction therapy consisted of dexamethasone (Dex), vincrstine (VCR), daunorubicin, asparaginase, etoposide (VP-16), aracytin (Ara-C), in addition to triple intrathecal (IT) therapy for CNS prophylaxis. Consolidation therapy included 2 courses of high dose methotrexate (HDMTX), 6-mercaptopurine (6-MP) and triple IT therapy. Continuation therapy consisted of

extended triple IT therapy and 15 cycles of 8-week course of VP-16+cycophosphamide (CTX), 6-MP+MTX, MTX+Ara-C, dexamethasone (Dex)+VCR, VP-16+Ara-C, 6-MP + HD-MTX, VP-16+Ara-C, Dex+VCR. A reinduction phase for 6 weeks was given starting on week 16 of continuation therapy and consisted of VCR, daunorubicin, asparaginase, Dex, HD-MTX, 6-MP and triple IT therapy. HDMTX was replaced by MTX IV or IM after week 53. VP-16 was replaced with oral 6-MP for 7 days after week 54 to minimize late drug effect.

Triple IT was given every 8 weeks during continuation therapy and discontinued after week 53 (first year of continuation treatment). Only patients with CNS leukemia at diagnosis and those with higher risk for CNS relapse (WBC count of 100 x 10^9/L or more) received IT therapy every 4 weeks during continuation therapy, followed by cranial irradiation at week 56 of continuation therapy. Cranial irradiation was given during weeks 56 to 59 to patients with high-risk for CNS relapse (18Gy) or CNS leukemia at diagnosis (24Gy).

Clinical evaluation and follow-up:

Bone marrow aspiration was done to evaluate response to chemotherapy at day 14 of induction therapy (early response) and day 43 (status post induction). Evaluable cases included 51 T-ALL. Cases who died before treatment or their data were not available were excluded.

Evaluable patients were followed up to evaluate disease status for a period ranging from 9-33 months with median observation period of 14 months.

Complete remission (CR) was defined as a normocellular BM containing less than 5% blast cells and showing evidence of normal maturation of other marrow elements.

Statistical analysis:

Patient data were tabulated and processed using SPSS for Windows [25]. Qualitative data are expressed as frequency and percentage, quantitative data as mean ± standard deviation and median. The Student *t* test and the chi-square test were used for comparative analysis. For 2 x 2 contingency tables, the Fisher exact test was used. Differences were considered significant at a p value of 0.05 and highly significant at a p value of 0.01 [25].

Life-table estimates were calculated using the Kaplan-Meier method, and the standard error of the life-table estimates was calculated with the Greenwood formula. Patients without adverse events were censored on the date of the last reported contact. The differences between curves were tested for statistical significance using the log rank test.

RESULTS

HOX11 gene family expression analysis:

RT-PCR was performed to detect HOX11L2 and HOX11 expression in 105 pediatric ALL cases, a cohort of 65 pediatric patients with T-ALL, 20 with B-ALL and 20 with AML. No specific fragment could be amplified from B-ALL, or AML samples, whereas a single 244-nucleotide fragment was observed in 8 of 65 (12.3%) T-ALL samples when tested for HOX11L2 expression. The expression of HOX11 was observed in 2 of 58 (3.4%) T-ALL samples tested, also detected as a single 244-nucleotide fragment. None of the HOX11L2 positive cases tested (7/8) expressed HOX11 as well. Patients' description is shown in Tables 3 and 4. PCR products of some HOX11L2 positive cases are presented in Fig. (1).

T-ALL cases were mostly compartment II (T intermediate, Ti) [32/65, (49.2%)], 26.2% in compartment I (T early, Te) and 24.6% in compartment III.(T late, Tl) Phenotype was heterogeneous for HOX11L2 (3 in compartment I, 2 in compartment II and 3 in compartment III).

Clinical outcome and follow-up:

Statistical analysis showed no statistically significant correlation between the HOX11L2 expression and age (median 10.5 years and 9 years for positive and negative cases respectively, p=0.38), gender (males 62.5% and 73.8% respectively, p=0.5) and total leucocytic count (TLC) (median 122.5x10^9/l and 121x10^9/l respectively, p=0.44). As for immunophenotyping the only statistically significant correlation (p=0.05) was seen between HOX11L2 expression and CD8. CD8 was expressed on 2/8 HOX11L2 positive cases irrespective of their maturation compartment, (1/2 T intermediate and 1/3 T late). Also it was expressed on. 1/3 T early but was 19% and in absence of surface CD3 the case was typed T early. We only had 2 cases Tl cytotoxic (ctx) and one of them expressed HOX11L2.

Six out of 8 HOX11L2 positive and 45/57 HOX11L2 negative cases were evaluable. All 6 evaluable HOX11L2 positive cases had BM less than 5% blasts on day 14 of induction therapy. The remission rates were comparable in HOX11L2 positive and negative patients. Table (5) shows status post induction of evaluable patients.

Event free survival (EFS) (Fig. 2) estimated at 3-years was 60%±22% for HOX11L2 positive and 70.6%±7.3% for HOX11L2 negative (p=0.59, log rank).

HOX11L2 as a marker for minimal residual disease (MRD) monitoring:

Because HOX11L2 expression is absent or barely detected in normal hematopoietic tissues **[12,16]**, HOX11L2 expression was tested in bone marrow samples from 3/8 cases during follow-up (Table 3). BM samples were collected in complete haematologic remission. Samples collected from patients 3 and 5 during CR at 1, 2 and 8 months after diagnosis showed no band at the expected site of HOX11L2. Three Samples were analyzed from patient 8. A sample at the time of lymph node relapse which occurred 9 months after diagnosis and although bone marrow was in CR (3% blasts) it showed a band at 244 base pairs (bp), the expected size of HOX11L2. A bone marrow sample was analyzed 11 months after diagnosis. It showed bone marrow relapse (blasts 57%) and HOX11L2 expression. HOX11L2 expression was detectable in the bone marrow 2 months before the frank bone marrow relapse. The patient received intensive therapy that included Fludarabin/Aracytin/Doxorubicin and bone marrow blasts went down to less than 5% blasts with persistent cervical lymphadenopathy and splenomegaly (no samples were available for molecular testing) but a second medullary relapse (BM blasts 45%) occurred 5 months after the last sample analyzed (16 months after diagnosis) and was again positive for the HOX11L2 expression (Fig. 1).

We came across another case that showed no HOX11L2 expression at presentation but the expression was detected at relapse. The patient was a male, 15 years old, TLC 354x10^9/L and T early phenotype. He went into CR for a year, and then he had BM relapse with HOX11L2 expression.

Table (1): Characteristics of T ALL patients with HOX11L2 and HOX11 status.

	T ALL cases	HOX11L2 positive	HOX11 positive
No. cases	65	8/65 (12.3%)	2/58 (3.4%)
Gender:			
Male	48 (72.3%)	5	2
Female	17 (27.7%)	3	0
Age at diagnosis (years):			
Median	10	10. 5	
Range	5mth-17y	3-16 y	9 and 14y
Low risk age group (1-9.99 yrs)	31 (47.7%)	3	1
High risk age group (<1 and 10 yrs)	34 (52.3%)	5	1
TLC count (x10^9/l):			
Less than 50	20 (30.8%)	2	1
50-100	10 (15.4%)	1	0
>100	33 (50.8%)	5	1
Ipt of T lineage:			
Te	17 (26.2%)	3 (37.5%)	1
Ti	32 (49.2%)	2 (25%)	1
TL*	16 (24.6%)	3 (37.5%)	0
Th	8	1	0
Tctx	2	1	0

*Cases expressing surface CD3 were considered T late (TL) however some of them did not express CD4 or CD8 (6/16, 37.5%).
Ipt=Immunophenotype. Te=T early. Ti=T intermediate. Th=T helper. Tctx=T cytotoxic.

Table (2): Panel of monoclonal antibodies (MoAbs).

Monoclonal Ab	Clone	Source
Myeloid Markers:		
CD13	My7-PE	Coulter Hialeah, FL
CD14	RmO52 PE	Coulter Hialeah, FL
CD33	M9-PE	Coulter Hialeah, FL
Lymphatic Markers:		
B Lineage		
CD19	BL6-FITC	Immunotech Marseille, France
CD22	Sd10 PE	Immunotech Marseille, France
T Lineage		
CD1	BL6	Coulter Hialeah, FL
CD2	39C1.5 FITC	Coulter Hialeah, FL
CD3	UCHT1 FITC	Coulter Hialeah, FL
CD4	13B8.2-FITC	Immunotech Marseille, France
CD5	BL1A-PE	Coulter Hialeah, FL
CD7	3A FITC	Coulter Hialeah, FL
CD8	B9.11 - PE	Immunotech Marseille, France
NK		
CD16	3G8 FITC	Coulter Hialeah, FL
CD56	N901 (NKH-1) PE	Coulter Hialeah, FL
Others:		
CD45	Immu19.2-FITC	Coulter Hialeah, FL
HLA-Dr	B8.12.2 FITC	Immunotech Marseille, France
CD10	d5 FITC	Coulter Hialeah, FL
CD34	581	Immunotech Marseille, France
Isotypic Controls:		
IgG1 (Mouse)	FITC	DAKO/COULTER/DIACLONE
IgG1 (Mouse)	PE	DAKO/COULTER/DIACLONE
IgG2a (Mouse)	FITC	DAKO/COULTER/DIACLONE
IgG2a (Mouse)	PE	DAKO/COULTER/DIACLONE

Table (3): Characteristics of T ALL patients with HOX11L2 expression.

No.	Sex	Age at diagnosis (yrs)	WBC count ($x10^9/l$)	Ipt of T lineage	MRD +ve/no tested	BMd14	Induction Response	Status
1	M	11	200	T early	0/0	NE	NE	Non evaluable
2	M	8	115	T early	0/0	M1	Died	Died induction
3	F	15	6	T early	0/1	M1	CR	Alive, CR
4	F	10.5	3.8	T int	0/0	M1	CR	Alive, CR
5	F	16	293	T int	0/1	M1	CR	Alive, CR
6	M	7	85.6	TLh	0/0	M1	Died	Died induction
7	M	3	220	TLctx	0/0	NE	NE	Non evaluable
8	M	16	188	TL	3/3	M1	CR	Relapse

+ve = Positive.
BMd14 = Bone marrow day14.
CR = Complete remission.
M1 = <5% blasts in BM.
NE = Non evaluable.
M = Males.
F = Females.

Table (4): Characteristics of T ALL patients with HOX11 expression.

No.	Sex	Age at diagnosis (yrs)	WBC count ($x10^9$/l)	Ipt of T lineage	MRD	BMd14	Induction Response	Status
A	M	14	31	Te	0	M3	CR	Alive, CR
B	M	9	136	Ti	0	M1	CR	Alive, CR

Te = T early.
Ti = T intermediate.
CR = Complete remission.
NE = Non evaluable.
M1 = <5% blasts in BM.
M3 = >25% blasts in BM.
M = Males.
F = Females.

Table (5): Status post induction of evaluable patients.

Status post†induction	HOX112+ (n=2)	HOX11L2– (n=45)	*p* value
Complete†remission	4/6 (66.6%)	33/45 (73.3%)	
Remission†failure	0/6	5/45 (11.1%)	0.88
Died	2/6 (33.3%)	7/45 (15.6%)	

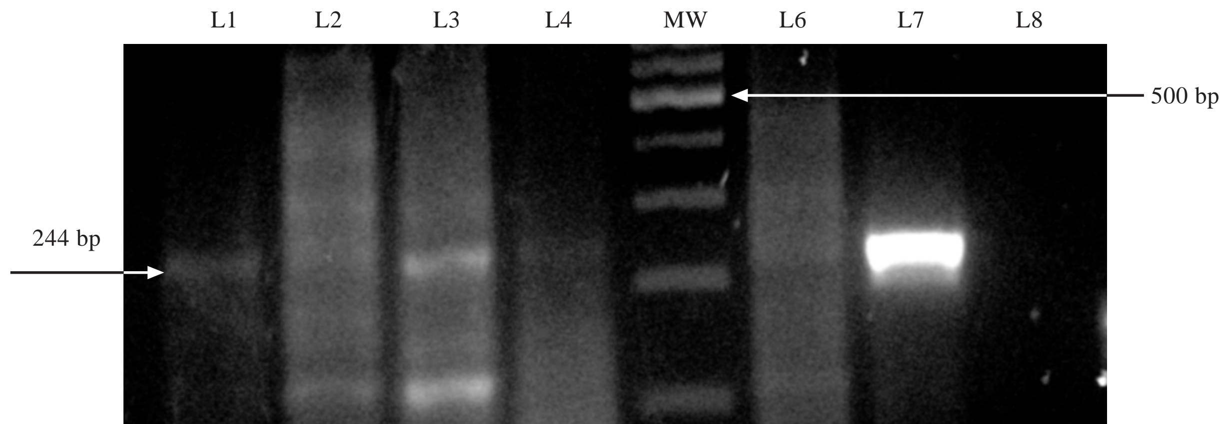

Fig. (1): PCR products of some cases tested for HOX11L2 expression, positive band 244bp.

L1: Follow-up sample of patient (8) 9 months after diagnosis.
L2 and L8: Are negative and template negative controls respectively
L3: BM relapse of patient (8) 2 months later, (11 months after diagnosis)
L4 and L6: Are follow-up BM samples for patients 3 and 5 in CR.
L5: 100bp MW marker
L7: Second BM relapse of patient (8) (16 months after diagnosis).

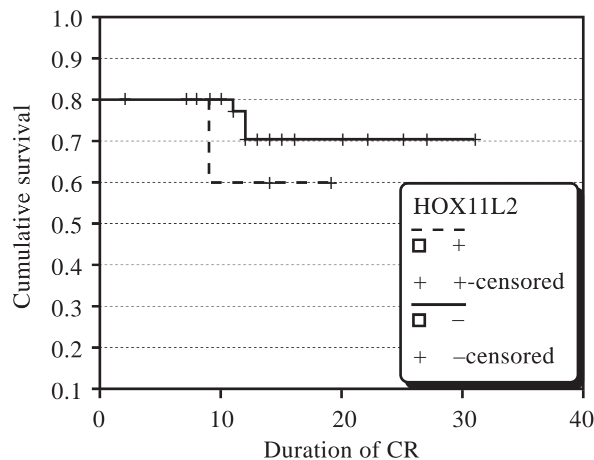

Fig. (2): EFS of studied patients.

DISCUSSION

T ALL comprises 26% of pediatric ALL in Egypt. In a study of T-cell compartments: 54%, 36% and 10% of patients had a phenotype of early (Te), intermediate (Ti) and late (Tl) T-cell maturation respectively (26). Early intrathymic compartment patients had 3-year probability of disease free survival (DFS) of 38% versus 62% and 100% for intermediate and late intrathymic compartments respectively [2]. In contrast, T-ALL cases in this study were mostly compartment II (49.2%), 26.2% in compartment I and 24.6% in compartment III.

T-ALL is infrequently characterized by chromosomal translocations, although several T-cell-specific genetic alterations have been described that correlate with different stages of thymocyte differentiation. Translocations t(11;14), t(10;14) and t(1;14) are the most common [12] with the most frequent recurring breakpoints within the 14q11, 7q32-q36 and 7p15 bands, which contain the T-cell receptor (TCR) genes TCRα/δ, TCR*β* and TCRγ, respectively [3-6].

The cryptic translocation t(5;14) (q35;q32) seems to be restricted to T-ALL. Analysis of t(5;14) (q35;q32) by FISH and/or of its molecular consequence HOX11L2 ectopic expression has been reported to represent the most frequent genetic alteration (24%) found in childhood T-cell leukemias so far [12,17]. However in the pediatric T-ALL patients studied in this work the HOX11L2 expression was detected in only 12% of cases. It is note worthy that the age of patients seems to be an important point. It has been shown that HOX11L2 deregulation decreased with age [12]. In a study of 153 cases the median age was 8 years with an age range of the 6-9 years at diagnosis and an incidence 23% in the HOX11L2-positive cases [17]. The age of the cases in this study was older (median of 10 years) with an age range of 3-16 years at diagnosis in the HOX11L2-positive cases.

HOX11L2 expression was reported to have strong correlation with male gender in one study [27]. However in our series, no association was observed with gender which is in concordance with other series that showed no significant difference in gender predilection [17].

HOX11L2 expression had no significant association with TLC count at diagnosis in this study as well as in other studies [17,27]. In a study all relapse events were observed in the group of patients with high TLC counts (>50x10^9/l) at diagnosis. Interestingly, all patients of this group who expressed HOX11L2 had relapses [16]. In our study, 6 patients with HOX11L2 had TLC count (>50x10^9/l), of which 2 were non evlauable, 2 died in induction, 1 haematologic relapse and one alive in CR.

Various studies reported that the phenotype was rather heterogeneous in the HOX11L2 group with two thirds (66%) having a cortical-T immunophenotype (CD1a$^+$) and 25% of the cases displaying a mature-T phenotype [17]. Some suggested that HOX11L2-expressing cases might define a T-ALL subgroup which shows a constant expression of CD1a and CD4 [15,28]. In this study the HOX11L2-expressing cases showed 2 cases (25%) with cortical-T immunophenotype and both (CD1a$^+$) and 3 cases (37.5%) mature-T phenotype with a statistical correlation with CD8 (p0.05). It is noteworthy to point out that there were 2 cases of T cytotoxic phenotype out of the 16 T late cases tested and one of them was HOX11L2-positive. However, CD8 expression was also found on a Te and a Ti HOX11L2-positive case.

Reports showed that the incidence of HOX11L2 decreased with age whereas that of HOX11 increased [12]. The impact on prognosis remains controversial; some reported HOX11L2 expression in pediatric T-ALL to be associated with poor prognosis [16]. Others did not confirm the unfavorable outcome [17].

In a study including 28 T-ALL cases, 6 cases were HOX11L2 positive and 4 of those patients developed relapse and hence appeared to have poor prognosis [16]. However in the much larger series (153 cases) the clinical outcome of patients from the HOX11L2 group was similar to that of the patients who did not display this abnormality [17]. The ectopic expression of HOX11L2 is probably not associated with the poor prognosis previously reported by other studies [16,17]. In concordance, this study showed no significant correlation between HOX11L2 expression and the clinical outcome, however, the number of HOX11L2 group was not big enough to draw conclusions.

The case reported in this study that was originally HOX11L2 negative and then expressed it when relapsed raises again the question about relapse whether it is a simple recurrence of the same disease or a development in the disease appearing due to further genetic events.

Some studies observed high levels of HOX11 transcript in 13% (8/59) of pediatric patients with T-ALL [14], while others reported it in only 7% (9/127) of cases [17]. Reports stressed on the association of the increased incidence of HOX11 expression with age [12]. However, the study that reported HOX11 expression to be in 7% of cases showed 2 peeks for the incidence

one at the age range 2-5 years and another above 10 years [17]. HOX11 over expression seems to be associated with a favorable outcome [14,29].

The 2 HOX11 positive cases reported in this study were 9 and 14 years old and both maintained CR. Because of the rarity of this genetic alteration, all studies lacked the statistical power that would have allowed any definitive conclusion about its prognostic significance. Study of a larger number of cases is required to establish whether the seemingly better outcome associated with t(10;14) and/or HOX11 over expression is indeed real [17].

The presence of occult disease in cancer patients after therapy is one of the major problems facing oncologists. Although 95% of pediatric T-cell acute lymphoblastic leukemia (T-ALL) patients have a complete therapeutic response to multiagent chemotherapy, half would relapse. These relapsing patients must have carried undetected MRD while in remission. The term MRD indicates the presence of leukemic cells below the detection level of conventional methods, i.e., $<10^{-2}$ leukemic cells [30]. Multivariate analysis showed that MRD information is an important prognostic factor at all follow-up time points taken during treatment and that this MRD information is independent of the classical clinical parameters at diagnosis such as age, gender, TLC, immunophenotype, chromosomal aberrations, and prednisone response. T-ALL is still heterogeneous in treatment response, but MRD information during treatment of these leukemia subtypes is more discriminative in predicting treatment outcome [31].

Our data suggest that HOX11L2 expression might be considered a suitable marker for minimal residual disease follow-up. During follow up, positive cases in complete remission showed no HOX11L2 expression while the relapsing case showed HOX11L2 expression 2 months before frank hematological relapse. Similarly, Ballerini et al. (2002) reported that monitoring HOX11L2 expression could be useful to follow the clearance of leukemic cells during the early phases of treatment as one patient who maintained HOX11L2 expression had a relapse, whereas the other patient showed a quick drop in the expression of HOX11L2 and did not have a relapse for 10 months [16].

Individualization of ALL treatment might further improve outcome and long-term quality of life. This may be achieved through MRD studies that allow the sensitive detection of leukemic cells undetectable by normal cytomorphologic examination, thereby providing accurate information about the in vivo efficacy of cytotoxic treatment [31].

In Conclusion: Our T-ALL cases showed a comparable HOX11 expression as recorded in western countries. However, we recorded a lower incidence of HOX11L2 expression than that in western countries. HOX11L2 expression may be used as a marker for minimal residual disease detection, however, further study on a larger scale is recommended.

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