



Fms-Like Tyrosine Kinase 3 expression in Childhood Acute lymphoblastic Leukemia at South Egypt Cancer Institute, Assiut University, Egypt

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Abstract

Background: FMS-Like Tyrosine kinase 3 (FLT3) plays an important role in early stages of hematopoiesis. FLT3 stimulation enhances proliferation and reduces apoptosis. One potential mechanism of FLT3 involvement in leukemia is over expression of its wild type and its ligand. The FLT3 protein is highly expressed in most patients with AML. In patients with ALL, FLT3 protein is highly expressed in up to 50% of leukemic blasts. Here, we aimed to evaluate the frequency of FLT3 protein expression in pediatric patients with ALL at South Egypt Cancer Institute, Assiut University, Egypt.

Method: FLT3 surface protein expression on leukemic blasts was detected by flowcytometry of 101 denovo pediatric acute lymphoblastic leukemia patients. High FLT3 expression considered when $\geq 20\%$ of malignant cells expressed CD135 and low FLT3 expression considered when $< 20\%$ of malignant cells expressed CD135. Relation between FLT3 expression and other clinical and laboratory findings were studied.

Results: High FLT3 expression was found in 47.5% of patients {39/74(52.7%) of precursor B-ALL and 9/27(33.3%) of precursor T-ALL}. High FLT3 level was significantly expressed in patients with the low risk age group ($p < 0.001$), patients who had no mediastinal mass, patients without lymphomatous features at presentation and patients with no CNS involvement at presentation ($p < 0.001$). 75% of patients with high FLT3 expression had TLC $< 50.000 \times 10^9$ ($p = 0.004$).

Conclusion: High FLT3 protein expression may be more commonly associated to favorable criteria of our ALL patients.

Background

FMS-like tyrosine kinase (FLT3, CD135) also referred to as fetal liver kinase 2 (flk-2) or stem cell tyrosine kinase (STK) located on chromosome 13q12 is a member of the class III receptor tyrosine kinase family that includes the c-kit, c-fms and platelet derived growth factor (PDGF) receptors (1). FLT3 is composed of four domains; the extracellular domains consisting of five immunoglobulin-like structures, trans-membrane (TM) domain, the juxta-membrane(JM) domain and the tyrosine kinase (TK) domain separated by a kinase insert(KI) followed by a carboxyl tail (2).

FLT3 plays an important role in early stages of hematopoiesis as it plays an important role in development of multipotent stem cells and B cells (3). In FLT3- expressing leukemic cells, FLT3 stimulation enhances proliferation and reduces apoptosis (4). One potential mechanism of FLT3 involvement in leukemia

is over expression of wild type FLT3 and its ligand, the existence of FLT3 activating mutations makes a much stronger case of the importance of this gene (5). The FLT3 protein is highly expressed in most patients with AML (6). In patients with ALL, FLT3 protein is highly expressed in up to 50% of leukemic blasts (7).

High FLT3 expression was an unfavorable prognostic factor for overall survival in AML patients without FLT3 mutations (7). In ALL, rare reports are available studying FLT3 expression and its relation to patient characteristic (8).

Several factors affecting prognosis; Age at diagnosis has strong prognostic significance as young children (aged 1 to < 10 years) have a better disease-free survival (DFS) than older children, adolescents, and infants (9). A total leucocytic count of $50,000/\mu\text{L}$ is generally used as an operational cut point between better and poorer prognosis (10). Children with ALL who present with

CNS disease at diagnosis are at a higher risk of treatment failure (both within the CNS and systemically) than patients with no CNS disease at diagnosis (11). Lymphomatous features and mediastinal mass are usually combined with a high TLC and a T-cell immunophenotype in the same patients (12).

Here, we aimed to evaluate the frequency of FLT3 protein expression in pediatric patients with ALL at South Egypt Cancer Institute, Assiut University, Egypt.

Methods

This prospective study was carried out on 101 newly diagnosed pediatric ALL patients who admitted at the Pediatric Oncology Department of South Egypt Cancer Institute, Assiut University, Egypt from April 2011 to august 2013 after ethical committee approval by our SECI IRB and informed consent from patient's family.

The study included 59 males and 42 females with a median age of 5 years at the time of diagnosis (range from 1-16 years). Diagnosis of the ALL patients was based on clinical, morphological, cytochemical, immunophenotypic and cytogenetic studies of lymphoblasts according to WHO classification. (13).

Detailed clinical history and examination were reported. Routine Laboratory investigations including complete blood count (CBC), liver function test(LFT), kidney function test, uric acid, electrolytes, cerebrospinal fluid (CSF) cytological examination and radiological imaging (chest X-ray (CXR) and magnetic resonance imaging (MRI) of brain) were obtained for all patients.

Detection of surface FLT3 receptor protein (CD135) expression on lymphoblasts was carried out on bone marrow (94 patients) and peripheral blood samples (7 patients) by flowcytometry. High FLT3 expression considered when $\geq 20\%$ of malignant cells expressed CD135. Low FLT3 expression considered when $< 20\%$ of malignant cells expressed CD135 as described by Peng et al and Vora et al (14, 15). Flowcytometric FLT3 protein expression detection is shown in **Figure (1)**.

Statistical analysis:

Statistical analysis was carried out using SPSS statistical software version 21. Qualitative data are expressed by frequency and percentage; quantitative data are expressed by mean \pm standard deviation and median. P value < 0.05 we considered significant.

Results

The median expression of FLT3 on leukemic blasts was 11.3 % with a wide variation of expression (range 0.07- 96.2%). High FLT3 protein expression ($\geq 20\%$) was found in 48 patients (47.5%) and low FLT3 expression was found in 53 patients (52.5%).

The majority of our pediatric ALL patients with high FLT3 expression (77.1%) was less than 10 years old at time of diagnosis, with TLC $< 50.000/mm^3$ (75%), without lymphomatous features (8.3%), without mediastinal mass (6.3%) and with no CNS involvement at presentation (4.2%) which was statistically

significant ($p < 0.005$) when compared with patients with low FLT3 expression. Precursor B-ALL represented (74, 73.3%) of patients and precursor T-ALL represented (27, 26.7%) of patients. High FLT3 expression represented (39/74) 52.7% of patients with precursor B-ALL and (9/27) 33.3% of precursor T-ALL ($p < 0.001$).

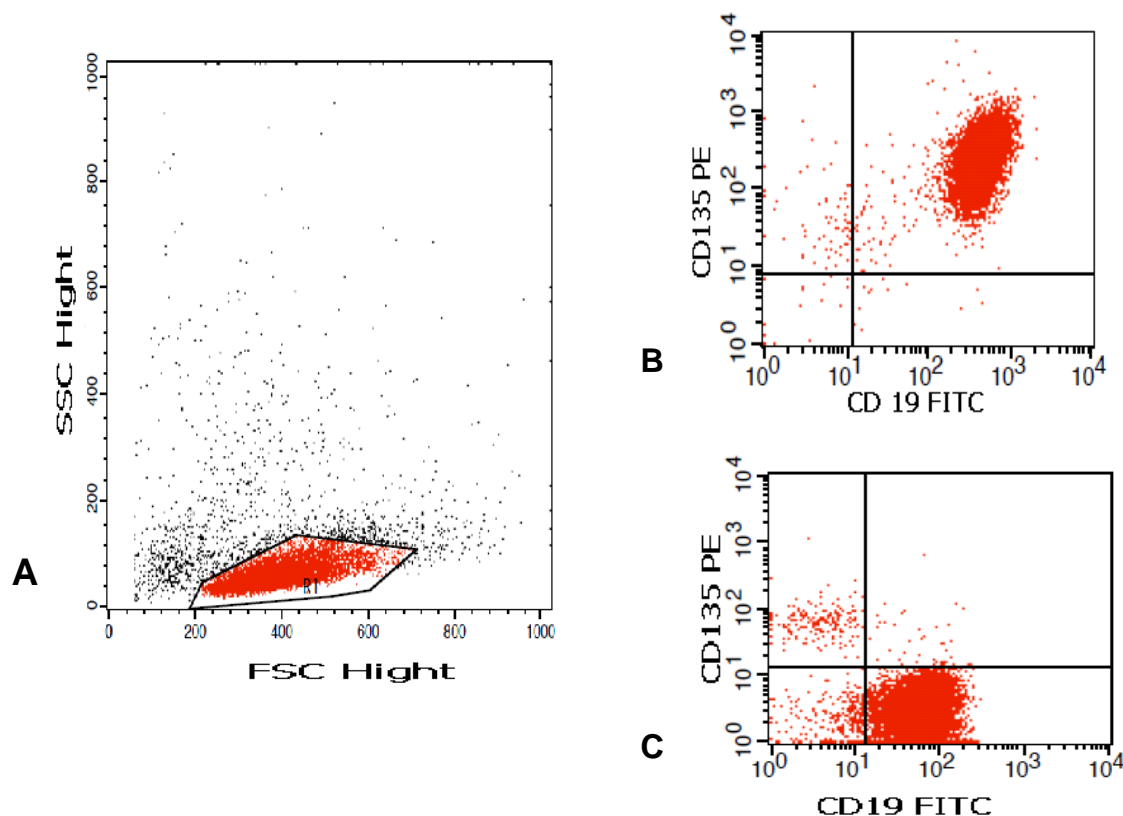
Table 1: Relation between FLT3 expression and other clinical and laboratory characteristics of 101 patients

	High FLT3 expression No. =48 (47.5%)	Low FLT3 expression No. =53 (52.5%)	P value
Age:			
<10ys	37(77.1%)	36(67.9%)	<0.001
≥ 10 ys	11(22.9%)	17(32.1%)	
Sex:			
male	25(52.1%)	34(64.2%)	0.185
female	23(47.9%)	19(35.8%)	
Lymphomatous features:			
Present	4(8.3%)	10(18.9%)	<0.001
Absent	44(91.7%)	43(81.1%)	
Mediastinal mass:			
Present	3(6.3%)	12(22.6%)	<0.001
Absent	45(93.7%)	41(77.4%)	
CNS involvement:			
Present	2(4.2%)	6(11.3%)	<0.001
Absent	46(95.8%)	47(88.7%)	
Total leucocytic count:			
$< 50.000 \times 10^9$	36(75%)	32(60.4%)	0.004
$\geq 50.000 \times 10^9$	12(25%)	21 (39.6%)	
Precursor B- ALL	39(81.3%)	35(66%)	<0.001
Precursor T-ALL	9(18.7%)	18(34%)	

Discussion

In our study, High FLT3 expression ($\geq 20\%$) was found in 47.5% of ALL patients which is similar to the results of **Ozeki et al., 2004** who demonstrated that up to 50% of pediatric ALL were highly expressed FLT3 protein (7).

High FLT3 protein expression ($\geq 20\%$) was found in 52.7% of our precursor B-ALL and 33.3% of precursor T-ALL. **Tarloc et al. 2013** reported that there is significant variation in FLT3 expression among pediatric population (16) which was reported in several studies as follow: 60% of precursor B-ALL and 23% of precursor T-ALL in pediatric (15), 64% of precursor B-ALL and less than 30% of precursor T-ALL (4) and 94% of precursor B-ALL, 32% of precursor T-ALL in pediatric (17, 18 &19). All these results including our result reported that FLT3 expression is more predominant in precursor B-ALL than in precursor T-ALL.



A: Forward and side scatter histogram was used to define the blast cells (R1).
The expression of CD135 was assessed in blast cells:
(B) High expression.
(C) Low expression.

Alavi et al 2013 reported that in AML, FLT3 expression may play a role in the survival or proliferation of leukemic blasts and it revealed poor prognosis (20). However, we found that high FLT3 expression was significantly correlated with most of favorable prognostic criteria as young age (<10ys), patients without mediastinal mass at presentation, patients without lymphomatous features at presentation, patients with no CNS involvement at presentation and patients with $TLC < 50 \times 10^9/L$. All of these finding can be explained by that FLT3 expression is more prevalent in precursor B-ALL but age ≥ 10 ys, mediastinal mass, lymphomatous features and CNS involvement and $TLC \geq 50 \times 10^9/L$ are more in T- cell phenotype. This can't be translated that FLT3 is a favorable factor. Also, no reports are available for us to compare our findings of these relations.

Different methods have been used to evaluate FLT3 expression levels include three methods for analysis FLT3 protein on cell surface membranes, flow cytometry (6, 7, 14, 21, 22), Western blotting (18, 23, 24), and ELIZA (25). Flow cytometry has advantage over Western blotting in protein estimation that was

reported by Wergeland et al (26). Reverse transcription-polymerase chain reaction (RT-PCR) was used to evaluate FLT3 mRNA levels in all leukemic cell lines (14, 18, 21). Many studies revealed that high FLT3 mRNA transcript level was noted in CD135 over expressing group and over expression of FLT3 mRNA along with FLT3 protein over expression was reported in AML.

Conclusion

High FLT3 protein expression may be more commonly associated to favorable criteria of our ALL patients but a larger number of patients are needed to confirm these results.

List of Abbreviations

AML	Acute Myeloid Leukemia
BM	Bone Marrow
TLC	Total Leucocytic Count
FLT3	FMS-like Tyrosine Kinase-3
FMS	Feline- McDonough Sarcoma
ALL	Acute Lymphoblastic Leukemia
CD135	Cluster of Differentiation135

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