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Evaluation of CD304 expression in pediatric B-lineage acute lymphoblastic leukemia

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Abstract:

BACKGROUND: Acute lymphoblastic leukemia is the most common form of cancer (25–30%) and predominant subtype of leukemia (75–80%) in children. However, childhood ALL has considerable phenotypic and genotypic heterogeneity, which is of diagnostic and prognostic importance. CD304 is a transmembrane glycoprotein C-type lectin found on plasmacytoid dendritic cells, It acts as a receptor for class III semaphorins mediating neuronal guidance and axonal growth.
Aim of the study: to study expression of CD304 in de novo newly diagnosed pediatric B- lineage ALL patients and to evaluate its association with hematological and clinical parameters.
Patients and methods: This cross sectional study was conducted on 30 pediatric patients with de novo newly diagnosed B-ALL randomly selected in relation to sex included (16 males and 14females) with their age ranging 1-14 year.
RESULTS: This study revealed that CD304 was detected in (56.67%) of newly diagnosed B-ALL patients and (43.33%) of them were negative for CD304 expression. It was detected that CD304 was not associated with age, gender, clinical presentations, other prognostic hematological parameters (Hb, WBC count, platelate count), blast percentage, NCI risk, B-ALL subtype, positive PAS and response to induction therapy.
CONCLUSIONS: CD304 expression was independent on the hematological and physical characteristics of patients, since the majority of positive CD304B-ALL cases were in high risk groups of NCI criteria and 60% of non- responder were positive CD304. PAS stain was detected in standard risk groups of NCI criteria, common B-ALL subtype and response to induction therapy.
Keywords: *CD304, Pediatric B-lineage ALL Patients, Flow Cytometry*

Introduction:

Acute lymphoblastic leukemia (ALL) is a malignant disorder resulting from expansion of immature lymphoid cells that originates in a single B- or T-lymphocyte progenitor from multistep somatic mutations in a single lymphoid progenitor cell at any stages of normal lymphoid development. (1) The patients are diagnosed on the basis of the clinical presentation, morphological and cytochemical evaluation of blood and bone marrow smears, together with immunophenotyping study. The diagnosis of acute leukemia was based on the presence of 20% or more blast cells in bone marrow (BM (2). B-lineage ALL is a hematological malignancy characterized by uncontrolled clonal expansion of B-lymphoid progenitors in the bone marrow (BM) and extramedullary sites. In childhood, it represents up to eighty percentage of the total diagnosed cases of acute lymphoblastic leukemia. The global incidence of BCP-ALL is 1–5 cases per 100,000 people.(3) CD304 (Neuropilin-1): is also known as BDCA-4, VEGF165R) is a 130 kDa trans-membrane c type lectin non-tyrosine kinase glycoprotein found specific for plasmacytoid dendritic cells in humans. It acts as a receptor for class III semaphorins (SEMA3s) mediating neuronal guidance and axonal growth.(4) Angiogenesis is an important requirement for the development and progression of hematological malignancies including leukemia and lymphoma.(5) CD304 expression is increased in hematological malignancies such as leukemia and lymphoma.(6,7) Therefore, NRP-1 could potentially be used as a target for ligand-directed therapy in leukemia and lymphoma.(8) Also, NRP-1 acts as useful marker to monitor minimal residual disease(MRD).(9) NRP-1 overexpression can be considered as a potential prognostic biomarker and associated with progression and unfavorable outcomes in patients with several types of cancers.(10)

Aim of the study: to study CD304expression in de novo newly diagnosed pediatric B- lineage ALL patients and to evaluate its association with hematological and clinical parameters.

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Patients, Materials and Methods:

This prospective cross sectional study was conducted from October 2018 to August 2019 in Central Teaching Hospital of Pediatrics, on approximately 30 de novo newly diagnosed pediatric B-lineage ALL patients before the start of chemotherapy ,randomly selected in relation to sex included (16 males and 14females) with an age at diagnosis ranging from 1-14 years. The patients included were stratified according to National Cancer Institute (NCI) criteria were categorised into Standard Risk (SR) and High Risk (HR) groups. The SR group with WBC count <50 x 109/l, age (1-10) years and patients with WBC count ≥ 50 x 109/l , age ≥10 years stratified as HR group .(11-13) After B-ALL cases have been confirmed by using specific B-cell lymphoid markers (CD10, CD19, CD20 and cyto-immunoglobulin) were analyzed using 4 colors FCM (BD FACS) & gating was done with CD45/SSC in Medical city/Baghdad, About (0.5_1) ml (EDTA) peripheral blood or bone marrow samples were transferred in cool box to be investigated for the expression of CD304 and cytoIgM to classify B-ALL subtypes in the private laboratory/Baghdad by using eight color FCM (BD FACS CantoTM II) /USA and the device software based on (BD FACSDiva). The blast gating strategy included using dot plots of FSC/SSC, CD45 expression versus intracellular complexity (SSC). All patients re-evaluated morphologically at day 28 from the start of induction therapy for assessment of complete remission (CR) achievement (BM blasts should be less than 5%). (14)

Statistical analysis: SPSS® Software (version 23.0 for Linux®) was used to perform the statistical analysis for this study. Qualitative data were represented as numbers and percentages, while continuous numerical data were represented as mean ± standard deviation. P value of < 0.05 was considered statistically significant.

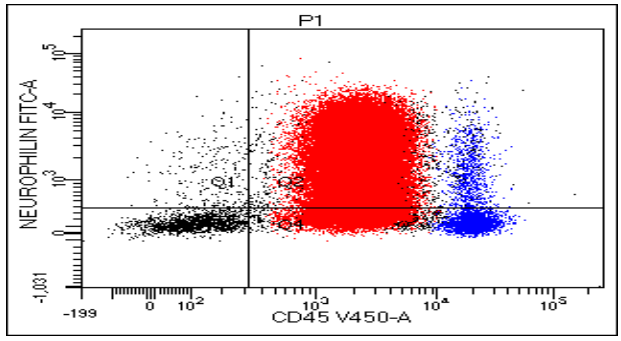

Figure 1: flow cytometric dot plot shows CD45V450/ CD304FITC

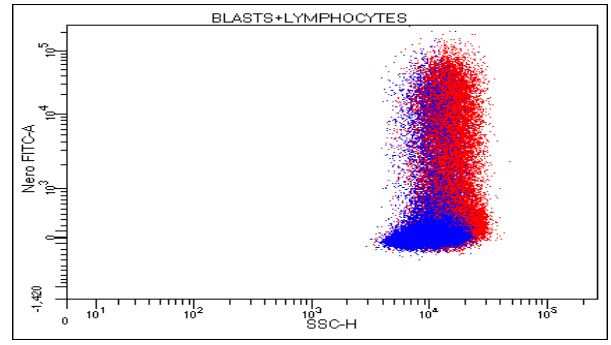

Figure 2: flow cytometric dot plot shows SCC/CD-304FITC

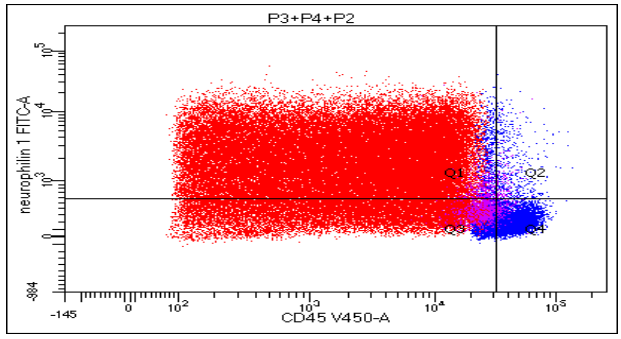

Figure 3: flow cytometric dot plot shows positive CD304 expression

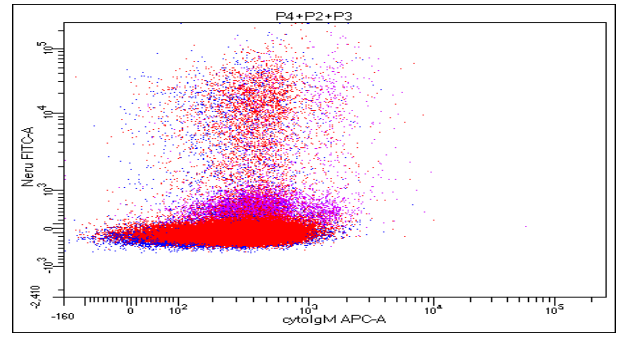

Figure 4: flow cytometric dot plot shows negative CD304 expression

Results:

The majority of patients (83.33%) were between (2-10 years) old with initial WBC count of less than 50 x109, these were two important parameters in stratification of patients according to NCI criteria, and two third of patients were in standard risk groups and one third were in high risk groups .Their age range was (2 -14 years) with a mean age of (6.17 ± 3.30 years) and a median of (5 years). Most of the patients (83.33%) were less than 10 years old while the remaining (16.67%) were 10 years or older, male patients forming (53.33%) of the study sample while female patients (46.67%). The majority of patients had pallor and fever (83.33% and 80.00%, respectively), and about three quarters of them (73.33%) had hepatosplenomegaly. Most of the patients had common B-ALL constituting 76.67% of B_ALL cases . About 13.33% of them had Pre B-ALL, while only 10 % had Pro B-ALL. Patients with positive CD304 formed (56.67%), while the remaining (43.33%) had negative CD304. There was no significant Correlation between CD304 with age, Student's t-test = 0.46 ,(P-value = 0.650) and gender (Figure1,2). There was no significant Correlation between CD304 and physical signs and symptoms, certain hematological parameters (Table:1,2) Also, No significant Correlation between CD304 status and risk group (Fisher exact P-value = 0.440), (Table-3) and B-ALL subtypes(Fisher exact P-value of 0.078). Also ,There was no significant correlation between CD304 status with PAS stain (Fisher exact P-value = 0.164) and response to therapy(Fisher exact P-value of 0.999). The standard risk Patients significantly responsed compared to high risk patients (Fisher exact P-value = 0.002),(Table-4).This study revealed all patients had negative Sudan Black B stain (SBB) and majority of patients (73.33%) had PAS stain was significantly associated with B-ALL subtypes(Fisher exact P-value = 0.032) and response to induction therapy(Fisher exact P-value of 0.044) ,(Table 5,6).There was no significant correlation between PAS stain and risk group(Fisher exact P-value = 0.478).

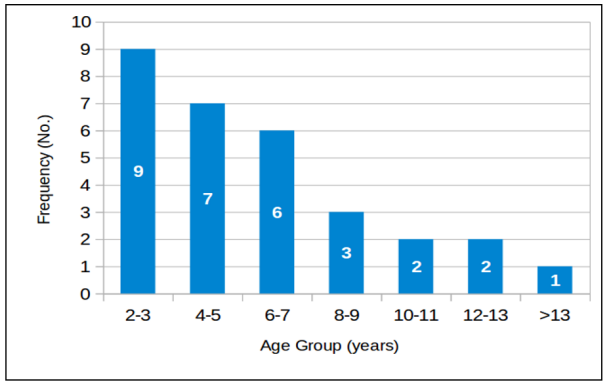

Figure-1: Age group distribution among study population

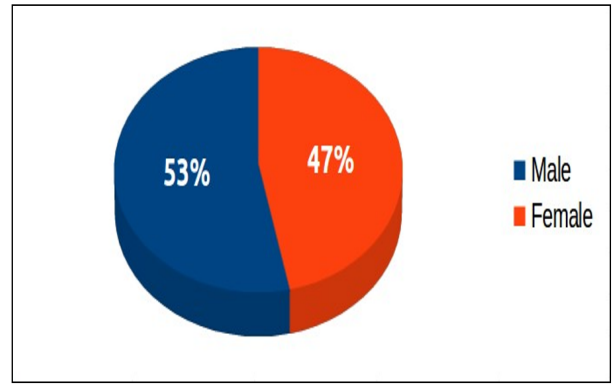

Figure -2: Gender distribution of the study population.

Table -1: Correlation between CD304 and physical signs and symptoms.

Symptoms and Signs		Status CD304		Total	value-P
Positive		Negative			
Fever	Positive	(58.33%) 14	(41.67%) 10	(100%) 24	*0.999
	Negative	(50.00%) 3	(50.00%) 3	(100%) 6	
Pallor	Positive	(52.00%) 13	(48.00%) 12	(100%) 25	*0.355
	Negative	(80.00%) 4	(20.00%) 1	(100%) 5	
Bruising	Positive	(50.00%) 10	(50.00%) 10	(100%) 20	*0.440
	Negative	(70.00%) 7	(30.00%) 3	(100%) 10	
HSM	Positive	(63.64%) 14	(36.36%) 8	(100%) 22	0.242
	Negative	(37.50%) 3	(62.50%) 5	(100%) 8	
LAP	Positive	(66.67%) 8	(33.33%) 4	(100%) 12	*0.367
	Negative	(50.00%) 9	(50.00%) 9	(100%) 18	
Pain Bone	Positive	(50.00%) 3	(50.00%) 3	(100%) 6	*0.999
	Negative	(58.33%) 14	(41.67%) 10	(100%) 24	

* Calculated using Fisher exact test ** Calculated using Chi-square test

Table-2: Correlation between CD304 status and hematological parameters.

Parameters	SD ± Mean		value-P
	CD304 Positive 17=n	CD304 Negative 13=n	
Hb (dL/g)	2.46 ± 7.82	2.05 ± 7.00	0.342
count Platelet (10^3×)	28.17 ± 41.86	77.82 ± 52.84	0.594
WBC (10^3×)	27.02 ± 28.38	16.90 ± 20.80	0.384
PBblasts (%)	21.69 ± 63.47	33.98 ± 42.28	0.064
blasts BM (%)	12.38 ± 87.24	26.21 ± 72.31	0.076

Table-3: Correlation between CD304 and NCI risk group

Group Risk NCI	Status CD304		Total	value-P
	Positive	Negative		
(SR) Risk Standard	10 (50.00%)	10 (50.00%)	20 (100%)	0.440
(HR) Risk High	7 (70.00%)	3 (30.00%)	10 (100%)	
Total	17 (56.67%)	13 (43.33%)	30 (100%)	

Table-4: NCI risk group correlation with response to induction therapy

Therapy Induction to Response	Group Risk NCI		Total	value-P
	(SR) Risk Standard	Risk High (HR)		
Response 25=n	(80.00%) 20	(20.00%) 5	(100%) 25	*0.002
response No 5=n	-	(100%) 5	(100%) 5	
Total	(66.67%) 20	(33.33%) 10	(100%) 30	

* Significant at P<0.05

Table-5: PAS stain correlation with the subtype of B-ALL

Stain PAS	Subtype ALL-B			Total	value-P
	ALL-B Common	ALL-B Pre	ALL-B Pro		
ve-	4 (50.00%)	1 (12.50%)	3 (37.50%)	8 (100%)	*0.032
(granules) ve+	6 (75.00%)	2 (25.00%)	-	8 (100%)	
(blocks) ve++	13 (92.86%)	1 (7.14%)	-	14 (100%)	
Total	23 (76.67%)	4 (13.33%)	3 (10.00%)	30 (100%)	

* Significant at P<0.05

Table-6: PAS stain correlation with response to induction therapy

Stain PAS	Therapy Induction		Total	value-P
	Response	response No		
ve-	5 (62.50%)	3 (37.50%)	8 (100%)	*0.044
(granules) ve+	6 (75.00%)	2 (25.00%)	8 (100%)	
(blocks) ve++	14 (100%)	-	14 (100%)	
Total	25 (83.33%)	5 (16.67%)	30 (100%)	

* Significant at P<0.05

Discussion:

The median age of de novo newly diagnosed B-ALL patients included in this study was 5 years old, comparable to many Iraqi and non-Iraqi studies. (15-17) The majority of the patients (83.33%) were between 2-10 years old while remaining (16.67%) of them were ≥10 years. Those results revealed that the majority of the patients were within favorable age group, comparable with many Iraqi and non-Iraqi studies. (15,16,18-20). The male to female ratio was (M:F 1.1:1), comparable to many Iraqi and non-Iraqi studies.(15,16,18). The majority of patients presented with pallor, fever followed by hepatosplenomegaly and bleeding tendency, and the least clinical features were lymphadenopathy and bone pain. Those results were similar to many Iraqi and non -Iraqi studies.(15,18,21,22). The mean of total leukocyte count was 25.10 ± 23.14.However(83.33) % of patients had WBC< 50 ×109 and only 16.67% of patients had WBC ≥ 50×109, this result agrees with many Iraqi and non-Iraqi studies.(15, 23) The CD304 expression was detected in 17 out of 30 (56.67%) of newly diagnosed B-ALL patients and (43.33%) of them were negative for CD304, its expression was lower than that revealed by Hagag and Nosair (5)(62%) and higher than that revealed by Sahar et al (30%).(22) The cut off value for positive CD304 expression on lymphoblast was 20% which is in agreement with many studies by Hagag and Nosair (5) , Karajalainen et al (7), Sahar et al (22), Meyerson et al (24), Younan et al (25), El Bordiny et al (26) and Abaza et al .(27) There was no significant association between CD304 expression with age , gender , physical signs and symptoms. those results were similar to Hagag and Nosair study (5) and El Bordiny et al (26) There was no significant association between CD304 expression and hematological parameters which similar to many studies (22, 26,27) also, there was no significant association between CD304 expression and the percentage of blasts in peripheral blood and bone marrow, this result was similar to many

studies.(28,29) This study revealed that half of SR patients had positive CD304 and the remaining (50%) of them were negative for CD304, whereas (70%) HR patients had positive CD304 and the remaining (30%) of HR patients had negative CD304,this result was similar with Mushtaq et al (21),Sahar et al (22) and Abaza et al (27). It was found all standard risk patients show response to induction therapy, whereas all non-responder were HR group.There was no significant association between CD304 status and B-ALL subtypes,this result was similar to Abaza et al.(27)The study revealed that 60% of non- responsive patients were CD304+ and 40% of non-responsive were negative CD304 but this correlation did not reach the level of significance and this result was in line with Abaza et al study.(27)

Conclusion:CD304 was expressed in 56.67 % of cases of de novo newly diagnosed childhood B-ALL. Its expression was independent on the hematological and physical characteristics of patients, since the majority of CD304+ B-ALL cases were in high risk group of NCI criteria and 60% of non- responder were CD304+. PAS positivity was detected in standard risk groups, common B-ALL subtype and response to induction therapy

Recommendation:Further studies with larger samples of patients and follow-up over longer time to determine the significance of CD304 as prognostic marker in B-ALL patients. Its Evaluation in relation with recurrent cytogenetic abnormalities include TEL-AML1 fusion gene and Philadelphia chromosome /BCR-ABL1 to confirm its expression with prognostic cytogenetic markers of ALL.

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