SMGroup

SM Journal of Hematology and Oncology

Research Article

Evaluation of CD 25 (IL2 Receptor Alpha) Expression in Adult Acute Lymphoblastic Leukemia Patients

Inas Ahmed Asfour[1], Gihan Mohamed Kamal Shams El Din[1], Rasha Magdy Mohammed Said[1]* and Entessar Mabrook Juadam[2]

[1]*Department of Internal Medicine and Clinical Hematology, Ain shams University, Egypt*
[2]*Department of oncology, Ain shams University, Egypt*

Article Information

Received date: Jul 18, 2017
Accepted date: Aug 14, 2017
Published date: Aug 22, 2017

***Corresponding author**

Rasha Magdy Mohammed Said, Department of Internal Medicine and Clinical Hematology, Ain shams University, Egypt, Tel: 02-01008226287; Email: rs_magdy07@yahoo.com

Distributed under Creative Commons CC-BY 4.0

Keywords ALL: Acute lymphoblastic leukemia; PH: Philadelphia Chromosome; FISH: Fluorescence in Situ Hybridization

Abbreviations ALL: Acute lymphoblastic leukemia; CBC: Complete blood count; FISH: Fluorescence In Situ Hybridization; PH: Philadelphia chromosome; ESR: Erythrocyte Sedimentation Rate; PBF: Peripheral Blood Film

BMA: Bone Marrow Aspirate; PCR: Polymerase Chain Reaction; EDTA: Ethylene Diamine Tetra Acetic Acid; WBcs: White Blood Count; Plt: Platelets; OS: Overall Survival; DFS: Disease Free Survival

Abstract

Background: Many parameters are included to determine the risk stratification of Acute Lymphoblastic Leukemia (ALL), Philadelphia Chromosome (Ph)/BCR-ABL–positive (ALL) is the largest genetically defined subtype in adult ALL with poor outcome. Here, we detected IL-2Rα (CD25) in patients with ALL and explored its diagnostic and prognostic value.

Patients and methods : Thirty ALL patients were recruited in Egypt , newly diagnosed with ages above 18 years old, after informed consent they invited to perform CD25 marker using Coulter EPICSXL, PCR for BCR – ABL fusion gene and Fluorescence in Situ Hybridization (FISH) were also performed along with CBC, LDH, Uric acid, CT scan allover and testicular ultrasonography.

Results: (70%) of patients were males while (30%) were females with no statistically significant difference as correlated with CD25, 13 (43. 33%) patients had positive CD25 , recurrent infections had occurred in 8 patients (26. 67%) with no significant correlation with CD25 (P = 0. 361), 16(53. 33%) patients suffered from fever, while 5 (16. 67%) experienced bleeding with no significant correlation among them with CD 25 (P> 0.05 in both). FISH cytology and PCR were positive in 11 (36.67%) patients. There was highly statistically significant correlation among CD25 and FISH and PCR for BCR-ABL, LDH, total leucocytic count with (P value <0.001). We showed that CD25 measurements compare favorably with other ALL prognostic criteria.

Conclusion: (CD25) expression was corresponding to Philadelphia chromosome. IL-2R α (CD25) is proved to be a valuable marker for monitoring ALL patients, an important parameter for prognosis and follow-up of ALL patients.

Introduction

Acute lymphoblastic leukemia is the most common type of cancer in children and adolescents accounting for 23% to 25% of all malignant diseases [1]

Philadelphia chromosome (Ph)/BCR-ABL–positive acute lympoblastic leukemia (ALL) is the largest genetically defined subtype in adult ALL with poor outcome. Philadelphia Chromosome-Positive (Ph+) Acute Lymphoblastic Leukemia (ALL) its Five-year overall survival rate ranges between 10-20% [2,3]**.**

Introduction of the Tyrosine Kinase Inhibitors (TKIS) in combination with chemotherapy have led to a marked improvement in treatment and the outcome of this category; with five -year overall survival now ranges from 40% to 50% [4].

Activated T-lymphocytes secrete IL-2 as lymphokine which plays a pivotal role in the growth and differentiation of T and B lymphocytes, monocytes and natural killer cells. The human IL-2 receptor (IL-2R) is a heterotrimeric complex consisting of α (CD25), β (CD122) and γ (CD132) chains. The CD25 chain is a transmembrane glycoprotein containing *N*- and O-linked glycosyl units with an apparent molecular weight of 55 kDa and is specific for IL-2 and increases the receptor affinity resulting in a drastic enhancement of T- lymphocyte proliferation. Recent data from crystal analysis suggest that the binding of CD25 to IL-2 stabilizes a secondary binding site for presentation to IL-2R -β chain, and CD25 has been used to distinguish a functionally relevant suppressive T-cell subpopulation [5]**.**

Expression of CD25 (interleukin-2 receptor alpha chain) by flow cytometric analysis has been shown to have a close association with Ph+ B-ALL in adult leukemia patients, studies suggesting that CD25 could be used as a surrogate marker for adult Philadelphia positive B acute lymphoblastic leukemia. Positive CD25 expression, using 25% as a cutoff, was observed in 6 of 8 (75%) Ph+ B-ALL patients (CD25+Ph+ B-ALL) with a median CD25 expression of 80.5% [**6**].

IL-2Rα levels were significantly higher in the ALL and AML patients than in the normal controls (P<0.05). In particular, the IL-2Rα level in the ALL patients was higher (2.561 ± 2.194 U/ml) than

the normal controls (421 ± 151U/ml) and most of them (92. 3%) had elevated plasma IL-2Rα levels [7].

CD25 expression was greater in Ph positive (Ph+) patients (80%) than in (Ph-) patients (17%), predicting Ph+ with 80% sensitivity and 86% specificity [8] .

In (Ph-) patients, CD25 expression (in 14 patients, 16%) was associated with RD (residual disease) in the cytogenetic intermediate-risk group: detectable RD in 50% of CD25+ patients versus 14% of CD25- patients. Interestingly, one CD25+/Ph- patient had a Ph-like genotype (ZC3HAV1-ABL2 rearrangement), and another had dual MYC-IGH and BCL2-IGH rearrangements [8].

This study aimed at a detailed assessment of the clinical implications diagnostic and prognostic value of the cluster of differentiation CD25 expression in newly diagnosed adult acute lymphoblastic leukemia patients to be correlated with various diagnostic and prognostic parameters of the disease.

Patients and Methods

This study was conducted on thirty adults patients at hematology / oncology department, Ain Shams University in Cairo, Egypt, patients were recruited from inpatient and outpatient departments with ages above 18 years old of newly diagnosed ALL during the years 2015 and 2016.

All patients were subjected at diagnosis to full clinical history and clinical examination, Laboratory investigations including Complete blood count & ESR, PBF, renal profile, liver function tests, LDH. BMA 2ml bone marrow were aspirated and put into EDTA containing tubes within 2 hours.

Immunophyno typing (flow cytometry) was performed on fresh blast cells according to standard protocol of WHO 2008 for ALL, CD25 marker was assessed for all patients .

Molecular Analysis

FISH and PCR for BCR-ABL fusion gene. CT-scan brain, neck, chest, abdomen and pelvic as well as Testicular ultrasonography.

Table 1: Showing the Expression of FISH, PCR for BCR-ABL, CD25 and the outcome of the patients included in the study.

		N	%
FISH for Ph	Negative	19	63.33
	Positive	11	36.67
	Total	30	100.00
PCR for BCR-ABL	Negative	19	63.33
	Positive	11	36.67
	Total	30	100.00
CD25	Negative	17	56.67
	Positive	13	43.33
	Total	30	100.00
Outcome after 6 months	Died	3	10.00
	Remission	14	46.67
	Relapse	13	43.33
	Total	30	100.00

The required sample size has been estimated using the Power Analysis and Sample Size software version 08. 0. 9 (PASS; NCSS; LLC; Kaysville, Utah). The test used for calculation is the two sided z-test and type 1 error has been set at a two sided value of 0. 05 (confidence level, 95%).

Results

Thirty patients with newly diagnosed ALL were recruited from clinical hematology unit at Ain shams university hospital over the period from 2015 and 2016 ranged from 16 to 70 years with Mean ±SD 35.471 in negative CD25, 34.769 in positive CD25 years and they were 21 males and 9 females.

Patients who previously received chemotherapy or in relapse are excluded from the study.

Impact of CD25 as diagnostic marker

Eleven out of the thirty patients (36.67%) were Ph (+ve) and PCR (+ve). CD25 was found to be positive in 13 out of 30, with (69. 23%) of the 13(+) were (Ph) positive and four patients of CD25 (+) were Ph (-ve) with high correlation with Ph chromosome in Table 1,2.

There were a highly statistical significant difference (P <0.01) between WBCs, FISH cytogenetics, PCR for BCR-ABL and CD25 and statistical significant difference (P<0.05) between serum LDH, the follow up and CD25. Serum lactate dehydrogenase reflecting the tumor burden transfer this character also to CD25 as a prognostic and predictor of the outcome .And these results encourage using this marker during the induction protocol as a guidance of the cure or relapse in Table 2.

Relationship between the CD25 and symptoms of ALL

There was no statistical significant difference as regard (P>0.05) age, sex, Hb level, Plt count, cellurity of marrow, Blast % and CD25. Anemic manifestations affected 13 (43. 33%) patients with no significant correlation withCD25 (P > 0. 05).Also recurrent infections had occurred in 8 patients (26. 67%) with no significant correlation with CD25 (P = 0. 361), 16 (53. 33%) patients suffered from fever, while 5 (16. 67%) experienced bleeding with no significant correlation with CD25 (P> 0.05) in both at diagnosis. Regarding the clinical picture of the disease, 27 (90.00%) of patients had hepatosplenomegaly, while 18 (60%) experienced adenopathy.

As regards the cellularity of the bone marrow, it was diluted in 2 (6.67%) patients, mild in 5 (16.67%) patients and hypercellular in 23 (76. 67%) patients. CD25 can be used during or in Nadir after chemotherapy which characterized by the anemic and bleeding manifestations.

ROC curve showed that CD25 got sensitivity (69%), specificity (88%), positive (82%) and negative predictive values (88%) with accuracy reaches up to (80%) in Figure 1 and Table 3. Concluded that CD25 expression is a specific and relatively sensitive marker for the identification of Ph+ B-ALL in the population. There were no statistical significant difference (P>0.05) between CD25 and the OS or the DFS in Figure 2 and 3.

Table 2: Showing the Correlation between CD25 and the age, sex of the patients, Comparison between the patients with positive and negative CD 25 regarding Hb, WBcs, Plt count ,uric acid, LDH, Cellularity of the marrow, blast% in marrow, FISH cytogenetics and PCR for BCR-ABL.
Outcome of patients after 6ms also was correlated to CD25.

		CD25				T-Test or Chi-Square	
		Negative		Positive		T or χ^2	P-value
Age	*Range*	18	77	19	52	0.127	0.899
	Mean±SD	35.471	18.361	34.769	8.428		
Sex	*Male*	13	76.47	8	61.54	0.782	0.376
	Female	4	23.53	5	38.46		
Hb	*Range*	6.1	14.7	6.5	13	0.110	0.913
	Mean±SD	9.129	2.479	9.038	1.898		
PLT	*Range*	2	250	15	824	-1.576	0.126
	Mean±SD	90.706	75.792	182.462	225.010		
WBC	*Range*	12	128.1	3.8	281	-3.833	0.001*
Uric acid	*Range*	3.4	6.5	3.1	15.5	-2.444	0.051
	Mean±SD	4.571	0.952	6.738	3.510		
LDH	*Range*	273	1380	440	1543	-2.655	0.013*
	Mean±SD	668.588	360.057	984.308	265.046		
Cellularity	*Diluted*	2	11.76	0	0.00	4.432	0.109
	Mild	1	5.88	4	30.77		
	Hypercellular	14	82.35	9	69.23		
Blast%	*Range*	42	98	60	98	-0.037	0.971
	Mean±SD	84.412	17.019	84.615	11.391		
Conventional Cytogenetics	*Range*	46	48	46	46	0.871	0.391
	Mean±SD	46.118	0.485	46.000	0.000		
FISH cytogenetics	*Negative*	15	88.24	4	30.77	10.476	0.001*
	Positive	2	11.76	9	69.23		
PCR	*Negative*	15	88.24	4	30.77	10.476	0.001*
	Positive	2	11.76	9	69.23		
Follow-up	*Died*	0	0.00	3	23.08	10.488	0.005*
	Remission	12	70.59	2	15.38		
	Relapse	5	29.41	8	61.54		

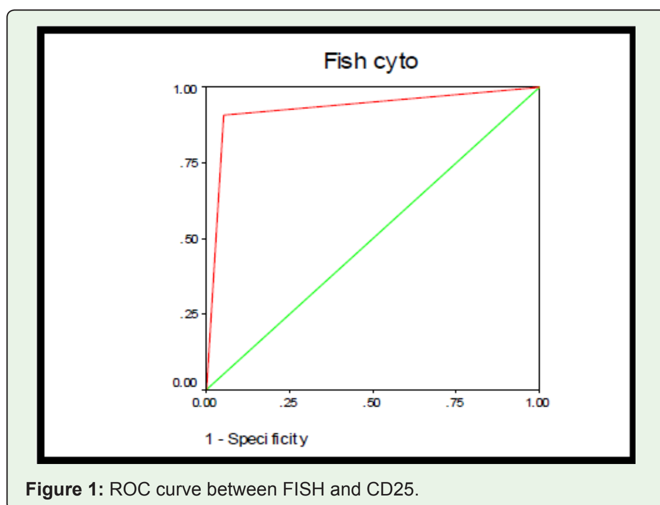

Figure 1: ROC curve between FISH and CD25.

Discussion

This study was designed to detect the close relationship and high sensitivity and predictive value of IL2Rα and its prevalence in ALL is considered as guidance towards diagnosis and as prognostic marker.

There was an agreement with study by Nakazawa et al [9,10,11] as regard CD25 positivity in ALL patients , who had earlier positivity of CD25 in all studied cases of ALL. A total of 88 newly diagnosed ALL patients were enrolled in it and (CD25) was detected by flow cytometry, and the expression of IL2Rα was detected by real-time qualitative RT-PCR, also BCR/ABL fusion gene was detected by qualitative PCR.

Their results showed that there was no statistical significant difference between WBC count and IL2Rα which disagreed with our results, Hb level, PLT count, marrow blasts percent (%), peripheral blast percent(%), hepato-renal infiltration, lymph node infiltration.

Table 3: Showing the Correlation between CD25 and the anemic manifestations, recurrent infections, fever, bleeding tendency. ROC cuvre has been showed the sensitivity, specificity, positive and negative predictive value and the accuracy of CD25.

	CD25						Chi-Square	
	Negative		Positive		Total			
	No	%	No	%	No	%	χ^2	P-value
Anemic manif								
Negative	10	58.82	7	53.85	17	56.67	0.074	0.785
Positive	7	18	6	46.15	13	43.33		
Total	17	100.00	13	100.00	30	100.00		
Recurr inf								
Negative	13	76.47	9	69.23	22	73.33	0.197	0.657
Positive	4	23.53	4	30.77	8	26.67		
Total	17	100.00	13	100.00	30	100.00		
Fever								
Negative	9	52.94	5	38.46	14	46.67	0.621	0.431
Positive	8	47.06	8	61.54	16	53.33		
Total	17	100.00	13	100.00	30	100.00		
Bleeding								
Negative	14	82.35	11	84.62	25	83.33	0.027	0.869
Positive	3	17.65	2	15.38	5	16.67		
Total	17	100.00	13	100.00	30	100.00		
	ROC Curve							
Sens.	**Spec.**		**PPV**		**NPV**		**Accuracy**	
69.23	88.24		81.82		78.95		80.0	

Positive BCR-ABL (+) was detected in (21/88) and their positive CD25 (+) expression level was 66.7% (14/21); 67 patients showed negative BCR/ABL(-) and their positive CD25(+) expression level was 4. 5% (3/67), there was statistical difference between these two groups (P <0.05) which agree with our results. Among 21 positive BCR/ABL (+) BALL patients the remission rate and relapsed rate were not statistical different between positive CD25 (+) and negative CD25 (-) groups which disagree with our results.

CD25 as mirror image to BCR-ABL with positivity of majority of ALL with Ph (+) predict the presence of BCR/ABL rearrangement in ALL. Also Kalina et al [12].who showed that in a cohort of 103 patients diagnosed with ALL detecting expression CD25 at presentation, CD25 was expressed by 33 cases (32%) . All BCR/ABL positive cases were positive for surface CD25 in agreement with our results [6].

CD25 was highly expressed in lymphoblasts in BCR/ABL-positive than BCR/ABL-negative cases of pre-B cell ALL which also observed by Cava et al [13].

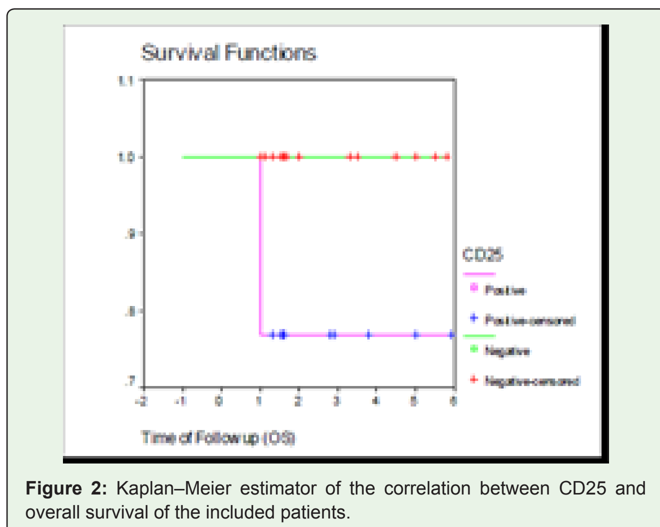

Figure 2: Kaplan–Meier estimator of the correlation between CD25 and overall survival of the included patients.

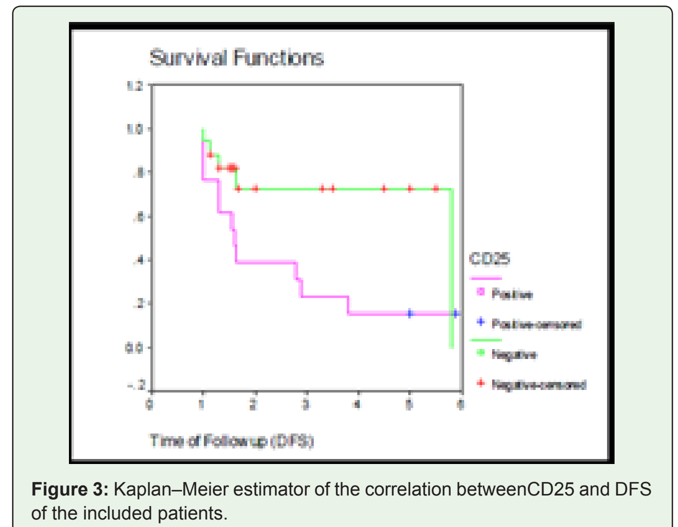

Figure 3: Kaplan–Meier estimator of the correlation betweenCD25 and DFS of the included patients.

Table 4: Showing the correlation between CD25 and OS and DFS.

CD25	OS			Log Rank	P-value
	Median	SE	95% Confidence Interval		
Negative	1.000	0.000	(1-1)	4.210	0.040*
Positive	4.770	0.570	(3.65-5.89)		
	DFS			4.810	0.028*
Negative	4.580	0.600	(3.41-5.75)		
Positive	1.600	0.200	(1.21-1.99)		

Our study performed a detailed assessment of the clinical implications and the prognostic value of the CD25, and CD25 assay is found to be a robust assay that is simple to perform, widely available, has a well-established interlaboratory reference range and is relatively in expensive [14].

Grimwad et al [15] and Cloos et al [16] both confirm the close relation of positivity of CD25 with FISH ,high levels of this receptor were closely associated with the expression of the B-lineage, CD13/33, CD34 and the presence of Ph chromosome [16] .

IL-2R which demonstrated a marked increase in ALL, on the cell surface of leukemia cells coupled to the increased sIL-2R could enhance leukemia progression by suppressing host antitumor immunity, and this may be one possible explanation for the leukocytosis (leukemic cell proliferation) and the dismal clinical course of IL-2R + ALL [17,18].

In our study CD25 was demonstrated to score high values of sensitivity and specificity and high positive and negative predictive marker and so CD25 expression is a specific and relatively sensitive marker for the identification of Ph+ B-ALL in the population [6].

CD25 expression may serve as a surrogate marker for BCR/ABL positivity (Philadelphia chromosome) [19], which is the major poor prognostic parameter in adult ALL. Another study did not describe a cutoff [19]. An arbitrary cut off of 20% to define cases as positive for CD25 expression was used by another [20], while yet another used 30% as a cutoff as determined by ROC curve [21] .

Lactate dehydrogenase enzyme activity (IU/L) of the included patients which is also a sensitive marker of tumor activity was found to be correlated with CD25 in agreement with Kornberg and Polliack [22].

CD25 is an independent prognostic factor in patients with ALL [23,24].

A correlation among CD25 and survival was found in detecting various stage of the disease either before initiation or 6 months after cessation of treatment [25,26].

In our study, Kaplan–Meier estimator of the correlation between CD25 and disease free survival (DFS) has revealed significant negative correlation reported that CD25 expression status improves prognostic risk classification in acute lymphoblastic leukemia [27].

Conclusion

The present study emphasized that (CD25) expression was in close relation to Philadelphia chromosome, PCR for BCR-ABL and reflecting the tumor burden and it differed according to the acute leukemia subgroups. IL-2R α (CD25) to be a valuable marker for diagnosis monitoring ALL before initiating or after chemotherapy. It represents highly important and informative parameter for prognosis and follow-up of ALL patients, revealed significant negative correlation with disease free survival.

References

1. Masetti R, Pesson A. First line treatment of acute lymphoblastic leukaemia with Peg asparaginese. Biologics. 2009; 3: 359-368.
2. Fluci V, Olombo T, Chiaretti S. Characterization of Band T-lineage ALL by integrated analysis of microRNA and mRNA Expression Profiles. Genes Chromosomes Cancer. 2009; 48: 1069-82.
3. Adele K Fielding. How I treat Philadelphia chromosome–positive acute lymphoblastic leukemia. Blood. 2010; 4: 116.
4. Oliver G, Heike P. Management of Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph+ALL). Hematology Am SocHematolEduc Program. 2009; 1: 371-81.
5. Qiao-Yan T, Ji-Yong Z, Jia-Jun W. Characterization of chicken interleukin 2 receptor α chain, a homolog to mammalian CD25. FEBS letter. 2006; 580: 4274-4281.
6. Amos SG, Rachel ED, Tarek ME. Original Article Expression of CD25 is a specific and relatively sensitive marker for the Philadelphia chromosome (BCR-ABL1) translocation in pediatric B acute lymphoblastic leukemia. Int J ClinExpPathol. 2014; 7: 6225-6230.
7. Yeonsook M, Yonggoo K, Myungshin K. Plasma Soluble Interleukin-2 Receptor (sIL-2R Levels in Patients with Acute Leukemia. Ann Clin Lab Sc. 2004; 34: 4.
8. Chen Q, Kim YC, Laurence A. IL-2 controls the stability of Foxp3 expression in TGF-β-induced Foxp3+ T cells in vivo. J. Immunol. 2011; 186: 6329-6337.
9. Mityushoa EV, Shatrova AN, Zenin VV. The STAT5 signaling in the expression of alpha-subunit of interleukin-2 receptor in human blood lymphocytes. Cell Tissue Biol. 2013; 7: 397-406.
10. Harlander RS, Liebler H, Mantler, SusHi: a program for the calculation of Higgs production in gluon fusion and bottom-quark annihilation in the Standard Model and the MSSM. Comput. Phys. Common. 2013; 184: 1605-1617.
11. Nakazawa HJ, Beier R, Loning L. Pharmacokinetics of native Escherichia coli asparaginase (Asparaginasemedac) and hypersensitivity reactions in ALL-BFM 95 re induction treatment. Br J Haematol. 2011; 114: 794-799.
12. Kalina T, Vaskova M, Mejstrikova E. Myeloid antigens in childhood lymphoblastic leukemia: clinical data point to regulation of CD66c distinct from other myeloid antigens. BMC Cancer. 2005; 5: 38.
13. Cava E, Kalina T, Trka J. Correlation of CD33 withpoorer prognosis in childhood ALL implicates a potential of anti-CD33 frontline therapy. Leukemia. 2000; 10: 1038.
14. Papadaki T, Iaccheri B, Fiore T. Serum soluble interleukin-2 receptor levels do not correlate with an associated systemic disease in patients with uveitis. Acta Ophthalmol. 2010; 88: 28-29.

15. Grimwad D, Walker H, Oliver F. on behalf of the Medical Research Council Adult and Children's Leukaemia Working Parties. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into the MRC AML10 trial. Blood. 1998; 92: 2322-2333.

16. Cloos J, Goemans BF, Hess CJ. Stability and prognostic influence of FLT3 mutations in paired initial and relapsed AML samples. Leukemia. 2006; 20: 1217-1220.

17. Wetzler M, Dodge RK, Mrózek K. Prospective karyotype analysis in adult acute lymphoblastic leukemia: the cancer and leukemia Group B experience. Blood. 2004; 93: 3983-3993.

18. Gleissner B, Gokbuget N, Bartram CR. Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. Blood. 2002; 99: 1536-1543.

19. Paietta E, Racevskis J, Neuberg D. Expression of CD25 (interleukin-2 receptor alpha chain) in adult acute lymphoblastic leukemia predicts for the presence of BCR/ABL fusion transcripts: results of a preliminary laboratory analysis of ECOG/MRC Intergroup Study E2993. Eastern Cooperative Oncology Group/Medical Research Council. Leukemia. 1997; 11: 1887-1890.

20. Nakase K, Kita K, Miwa H. Clinical and prognostic significance of cytokine receptor expression in adult acute lymphoblastic leukemia: interleukin-2 receptor alpha chain predicts a poor prognosis. Leukemia. 2007; 21: 326-332.

21. Jaso J, Thmas DA, Cunningham K. Prognostic significance of immunophenotypic and karyotypic features of Philadelphia positive B-lymphoblastic leukemia in the era of tyrosine kinase inhibitors. Cancer. 2011; 117: 4009-4017.

22. Kornberg A, Polliack A. Serum lactic dehydrogenase (LDH) levels in acute leukemia: marked elevations in lymphoblastic leukemia. Blood. 1980; 56: 351-355.

23. Greer, JP, Arber, D A, Glader, B. Wintrobe's Clinical Hematology (13th ed.). Lippincott Williams, Wilkins. 2013.

24. Skacel M, Ormsby AH, Pettay JD. Aneusomy of chromosomes 7, 8, and 17 and amplification of HER-2/neuand epidermal growth factor receptor in Gleason score 7prostate carcinoma: a differential fluorescent in situhybridization study of Gleason pattern 3 and 4 using tissue microarray. Hum Pathol. 2003; 32: 1392-1397.

25. Perdigao J, Cabrai MJ, Costa N. Prognostic factors in CLL: Is serum free light chain ratio a new biological marker? Annals of Oncology. 2008; 19: 204.

26. Ruchlemer R, Reinus C, Paz E. Free light chains,monoclonal proteins and chronic lymphocytic leukaemia. Blood. 2007; 110: 4697.

27. Gönen Mihat, Zhuoxin Sun, Maria E. Figueroa. CD25 expression status improves prognostic risk classification in ALL independent of established biomarkers: ECOG phase 3trial, E1900. Blood. 13; 120: 2297-2306.

Citation: Asfour IA, El Din GMKS, Said RMM and Juadam EM. Evaluation of CD 25 (IL2 Receptor Alpha) Expression in Adult Acute Lymphoblastic Leukemia Patients. SM J Hematol Oncol. 2017; 2(1): 1005s.