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Surrogate Role of CD85k on Monocytic Lineage Involved Leukemogenesis Biology and Clinical Aspect

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Abstract

Background: Unique receptor involved in leukemogenesis is CD85k; an immuneglobulin receptor for immune tolerance, CD36 is glycoprotein mediates cellular adhesion and metastatic spread, CD14, CD15 considered common monocytic markers. **Aims:** to investigate CD85k with monocytic lineage involved leukemia (MLIL) markers in leukemia pathogenesis and clinical presentation. **Patients and Methods:** 47 patients (32 diagnosed acute myeloid leukemia (AML); 15 non-malignant hematological disease as a control), were included, aged from 2 to 80 years, all subjected to peripheral blood (P.Bl) and bone marrow (B.M) examination, immunophenotyping (IPT) using FASC Canto four color flow cytometer (FCM) Becton Dickenson (BD) USA, for CD13, CD33, MPO, HLA-DR, CD34, CD38, CD117, CD14, CD15 and CD36 the Mo Abs supplied by B.D Bioscience, and anti CD85k Mo Abs by Aveda de Coimbra Flamenco, reference No. 1399990130. **Results:** Frequency of CD85k is 19/32 (59.37%) of AML; 14/14 (M4/M5) 100% positive CD85k, insignificant correlations of CD85k to sex, lymphadenopathy or organomegaly, platelets count and P.Bl blast ($P > 0.05$), significant to age < 35 years, WBCs > 50,000 × 10^9/l, Hb < 7 g/dl, BM blasts, CD34 and HLA-DR CD33, CD13, CD38 ($P < 0.05$), insignificant correlations to CD36, CD14, CD15 and CD117 ($P > 0.05$). **Conclusion:** Although CD85k is MLIL associated marker, it is not correlated with other MLIL markers with frequency 100% in MLIL and 59.37% in AML, age predisposition is <35 years with no sex variation, significant correlation to progenitor and myeloid markers, it's a crucial role in leukemogenesis biology, not in clinical presentations, considered good follow up predictor MLIL marker.

Keywords

CD85k, Monocytic Lineage Leukemia

1. Introduction

The unique synergistic action of membrane bound proteins in leukemogenesis in monocytic lineage involved (MLIL) leukemia include immune escape by immunoglobulin inhibitory receptor CD85k and cellular adhesion and metastatic spread by glycoprotein CD36 [1] [2]. CD36 mediating adhesion process to endothelial cells and in promoting tumor spreading and organ infiltrations [3]. Monocytoid dendritic cells with high ILT3 levels suppress T cell activation and are tolerogenic, ILT3 levels are higher in patients being treated with type I IFN, supporting the concept that IFN-induced ILT3 expression is immunosuppressive [1] [2] [4]. The cytoplasmic tail of ILT3 contains immune tyrosine-based inhibition receptor recruits and activates tyrosine phosphatases. Ligation of CD85k on dendritic cells blocks the activation and downstream signaling [5] [6] [7]. CD85k expressed by, monocytes, dendritic cells and endothelial cells. It is encoded in chromosome 19 [8]. CD85k has a crucial role in tolerogenic activity of antigen presenting cells and tumor escape [9]. Also it promotes conversion alloreactive CD4 to regulatory T cell (Terg). It inhibits T cell proliferation and induces CD8 differentiation. Crosslinking of CD85k to monocyte receptor decrease activation [10]. In AML CD85k is sensitive marker with CD36 for monocytic differentiation [3] [11]. In MLIL CD85k co-expressed with CD34 and CD117 progenitor cells so it has a role in leukemogenesis [10]. AML with monocytic differentiation has a high risk of extra medullary disease, high leukocytic count and coagulation defect also genetic and cytogenetic abnormality [8]. The early clinical findings of AML are often vague and nonspecific [12]. Splenic enlargement and generalized lymphadenopathy are rare in AML [13]. Some patients may experience swelling of the gums because of infiltration of leukemic cells [14]. AML has several subtypes; treatment and prognosis differ between them, several markers can predict which drug may work best [15].

CD85k mainly has prognostic value in leukemia so it should be incorporated into the initial diagnosis work-up and leukemia monitoring [9]. CD85k is an important target for anticancer therapy [10]. Lack of CD85k expression leads to leukemia remission increase survival rate in animal model, also block leukemia development in transplantation [16].

2. Subjects and Methods

2.1. Ethical Approval

The present study was revised by the Scientific Ethical Committee of Sohag University Hospital; a written informed consent was taken from all patients groups. It was in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration.

2.2. Patients Selection

32 newly diagnosed AML patients were rolled in the study, they attended to Sohag University Hospital, Hematology Unit of Clinical Pathology Department,

from October 2014 to October 2016, they were aged from 2 to 80 years old with mean age (31.77 ± 19.49), 19 males and 13 females and 15 subjects of non-malignant hematological disease (ITP), 8 males, 7 females; their ages ranged from 3 to 60 years with mean of (15.47 ± 15.46) of the same ethnic group as a control.

2.3. Inclusion Criteria

Newly diagnosed AML especially MLIL. All were subjected to:

Through history and clinical examination with stress on the presence and extent of leukemia involvement of liver, spleen, lymphadenopathy and gum hypertrophy.

2.4. Laboratory Investigations

2.4.1. Sampling

3 ml of venous blood were collected from each one, dispensed into (K-EDTA) B.D tube used for P.Bl hemogram. BM examinations (aspirate/biopsy) were done for all groups, diagnosis of AML based on morphological features of P.Bl and B.M smears, cytochemical tests and IPT data. Blood count using Cell-Dyne-Ruby, automated cell counter, ABBOTT diagnostic (USA), with microscopic examination of stained P.Bl. and BMA smears for differential leucocytes count, blast cells percentage, morphological features and cytochemical stains.

2.4.2. IPT of Blast Cells

FCM FASC Canto four colors B.D; USA was used and the MoAbs supplied by B.D Bioscience, USA. The panel of fluorescein isothiocyanate (FITC), phycoerythrin (PE) and Peridinin chlorophyll (PerCP) conjugated MoAbs were used for each sample. Common progenitor marker, CD34, HLA-DR, CD38, CD117 Myeloid markers CD13, CD33, MPO, monocytic marker CD14, CD15, CD36, CD65, CD68. Lymphoid markers: B cell markers CD19, CD22, CD10, T cell markers CD2, CD3, CD5, CD7, FITC labeled MoAbs for detection of CD36 (B.D Bioscience, Cat. No.656151 USA). PE labeled Mo Abs for CD85k provided by Aveda de Coimbra Flamenco, Reference No. 1399990130.

2.5. Procedure of Surface Membrane Markers

Expression on blast cells:

Reagents

Sheath fluid, Phosphate buffered saline (PBS) (8.0 g/L NaCl, 0.2 g/L k Cl, 1.15 g/L NaH_2PO_4 and 0.2 g kH_2PO_4) added to 100 mL of distilled water with pH adjusted at 7.3 ± 0.2. Lysing solution (1.5 mmol/L NH_4Cl, 100 mmol/L $KHCO_3$ and 10 mmol/L tetra Na-EDTA) made up to 1 liter with distilled water, pH adjusted at 7.2. Negative isotypic control (appropriately labeled according to the MoAbs used) for determining the non-specific binding of MoAbs. MoAbs supplied by BD Bioscience, United States.

2.6. Procedure

Blood was diluted with (PBS) so that WBCs count was adjusted between 5 and 10×10^9/l. For each sample, sets of tubes were labeled for all the MoAbs to be used, including 1 tube for the appropriate negative isotypic control.

50 µL of diluted samples were delivered in each tube. 5 µL of each MoAbs as well as of the isotypic negative control. The tubes were vortexed and incubated in the dark at room temperature for 15 minutes. The tubes were centrifuged at 500 rpm for 5 minutes and the supernatant was discarded. Lysing solution (1.5 mL) was added to each tube. The tubes were vortexed and incubated for 5 - 10 minutes in the dark at room temperature. 2 ml PBS was added and the tubes were vortexed. The tubes were centrifuged at 500 rpm for 5 minutes and the supernatant was discarded. Cells were suspended in 500 µL PBS to be ready for acquiring data by the FCM.

The expression of blast cells for CD85k was determined as a percentage from the gated blast cells population. Cells were considered positive for a certain marker when ≥20% of cells expressed it, except for CD34, cytoplasmic MPO and CD85K where its expression by 10% of cells was sufficient to confer positivity.

2.7. Statistical Analysis

The collected data were tabulated and analyzed using statistical package of social science (SPSS) version 17 software. Suitable statistical techniques were computed ANOVA test, Student's t test, Mann Whitney test and Kruskal Wallis for non-parametric values, correlation coefficient were used as tests of significance. Qualitative data were described in the form of number and percentage. Quantitative data were described in the form of mean ± standard deviation (SD), range and median.

2.8. Results

The present study was carried on 47 patients thirty two diagnosed as AML including fourteen MLIL (M4/M5 cases), their age ranged from 2 to 80 years, with median age 29 years old (mean is 31.77 ± 19.49), they were nineteen males/thirteen females, and fifteen cases of non-malignant hematological disease (ITP); their age ranged from 3 to 60 with median age 15 years old (mean 15.47 ± 15.46); seven males/eight females as a control.

Table 1 represents demographic data and clinical features in relation to CD85k expression, the positive rate of CD85k was 59.37% in AML and 100% in MLIL patients. In this study we notice that; the age predisposition of CD85k expression in AML patients was that; (12/19 cases) were below 35 years old and (7/19 cases) were above 35 years old (significant p value $p < 0.05$). About the sex variation, the number of AML males positive for CD85k was 10/19, while the positive females were 9/19 with insignificant difference ($p > 0.5$). Hepatomegaly was observed in 18/32 patients AML (56.25%) from which 12 patients were positive for CD85k (60%); the other fourteen patient with normal liver size showed

Table 1. Demographic data with clinical features and hematological variables in the studied patients.

Patients Variables	AML group	CD85k + Ve	AML subgroup (MLIL)	Control	p-value
Patients No	32		14	15	NS
Age (years)					
Median (range)	19 (2 - 80)		20(2 - 74)	15 (3 - 60)	NS 0.06
<35	14 (12)		10		S 0.03
>35	18 (7)		4		NS 0.08
Sex					
M/F	19/13	(10/9)	6/8	8/7	NS 0.5
Hepatomegaly	18 (12)		12	Normal	NS 0.3
Normal size	14 (7)		2		
Splenomegaly	20 (15)		12	Normal	NS 0.4
Normal size	12 (4)		2		
Lymphadenopathy	14 (10)		10	Normal	NS 0.2
No lymphadenopathy	18 (9)		2		
WBCs × 10^9/l				3.2 - 12.8	
Range	1.7 - 245		23 - 245		
<50	21		9		
>50	11		5		S 0.02
Hb g/dl					
Range	3.7 - 12.6		4.5 - 8	11 - 13.5	
<7	19		10		S 0.023
>7	13		4		
Platelets × 10^9/l					
Range	12 - 456		14 - 38	243 - 427	
<100	24		14		S 0.02
>100	8		0		
P.Bl. blasts %					
Range	11 - 91		14 - 69	0.0	NS 0.06
B.M blasts %					
Range	24 - 93		23 - 78	0.0	NS 0.5
CD85k + Ve					HS 0.001
% Range	(0.63 - 87.6)	(19)	(23 - 87.6)	0.0	
Mean ± SD	26.92 ± 25.29		14		

AML: acute myeloid leukemia, MLIL: monocytic lineage involved leukemia, M: male, F: female, P.Bl: peripheral blood, BM: bone marrow, WBCs: white blood cells, Hb: hemoglobin, CD cluster of differentiation, HLA: human leukocytic Ag.

50% positive expression of CD85k (7 cases). Splenomegaly was observed in 20/32 ML patients 62.5%, from which 15 patients were positive for CD85k (75%) and five were negative; and the other twelve patients with normal size spleen showed 30% positive for CD85k (4 cases). Lymphadenopathy was observed in 14/32 AML patients (43.75%); nine patients from them showed positive CD85k 9/14 (64.28%), and 10/18 patients without lymphadenopathy showed positive CD85k (55.55%). All these data were insignificant to CD85k expression ($p > 0.05$).

The positive rate expression of both CD 85k and other MLIL markers were zero within the control group. As regard the hematological variables, also in table-1; significant correlations of CD85k was found when WBCs count was more than 50×10^9/l ($p < 0.05$), Hb value was less than 7 g/dl ($p < 0.05$), also significant correlation to the percentage of BM blasts. While platelet counts, the P.Bl blasts showed insignificant correlations ($p > 0.05$). We found that; the number of AML cases positive for progenitor markers were seventeen patients were positive for CD34, thirty were positive for CD38, 6 cases were positive for CD117 and twenty four patients were positive for HLA-DR. While the number of positive cases for the rest MLIL markers were 6 patients were positive for CD14, 8 patients were positive for CD15 and only 3 cases were positive forCD36. Myeloid markers positivity showed that; CD13 was positive in twenty eight cases and CD33 was positive in thirty patients.

Table 2 represents the correlation of CD85k to the progenitors, myeloid and MLIL FCM markers. We notice that, there was insignificant correlation between CD85K and CD16, CD117, CD36, 235a.There was no correlation between CD85K and CD45, CD14 and CD15. There was positive significant correlation between CD85K and HLA-DR, CD34 and CD38, also CD13 and CD33 showed significant correlation. Details of FCM analysis were illustrated in Figure 1, Figure 2.

Comparison between MLIL and other types of AML as regards IPT were presented in Table 3, we found that the mean expression of CD45, CD13, CD33 was increased in MLIL than other AML group, with significant increase in CD45 ($p = 0.002$). On the other hand, the mean expression of MPO, CD38, CD117, CD34 was increased in other AML group than MLIL with significant p value in CD 34, CD38, CD117 (P0.01, 0.005, 0.011 respectively). While the mean expression of HLA-DR, CD14, CD15 showed significant increase in MLIL group than other AML group ($p < 0.001$) for all markers. The mean expression of CD36 and CD61 were higher in other AML group than MLIL group (***P*** 0.027 and 0.01 respectively). High significant increase in the mean expression of 235a in other AML group than MLIL group ($p < 0.001$). Finally the mean expression of CD85k is highly significant increase in MLIL group than other AML group (43.23 ± 18.46 to 14.23 ± 22.07); $p < 0.001$.

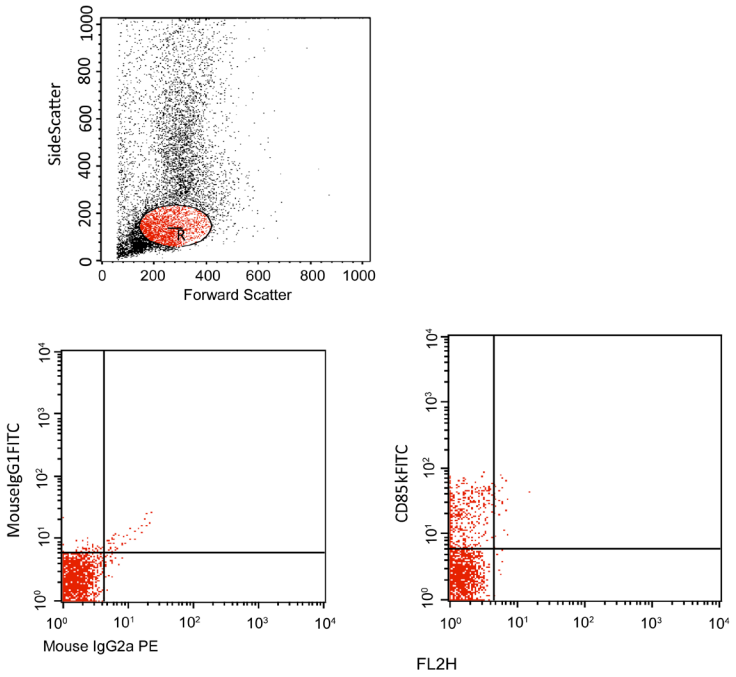
3. Discussion

Membrane bound protein receptors play a crucial role in leukemogenesis, as

Table 2. Spearmans' correlation of CD85k to clinical and hematological variables in AML patients.

FCM markers	Spearman's correlation	p value
Age	0.33	0.06 (NS)
Sex	0.137	0.09 (NS)
	WBCs	
$<50 \times 10^9$/l	0.111	0.148 (NS)
$>50 \times 10^9$/l	0.35	0.048 (S)
Hb < 7 g/dl	0.346	0.034 (S)
Hb > 7 g/dl	0.23	0.22 (NS)
Platelets	0.06	0.73 (NS)
P.Bl blasts	0.32	0.07 (NS)
B.M blasts	0.42	0.02 (S)
	Progenitors	
CD34	0.494	0.03 (S)
HLA-DR	0.544	0.004 (S)
CD38	0.471	0.035 (S)
CD117	0.0435	0.734 (NS)
	Myeloid	
CD13	0.399	0.011 (S)
CD33	0.457	0.003 (S)
	Monocytic	
CD14	0.267	0.26 (NS)
CD15	−0.363	0.12 (NS)
CD36	0.064	0.651 (NS)

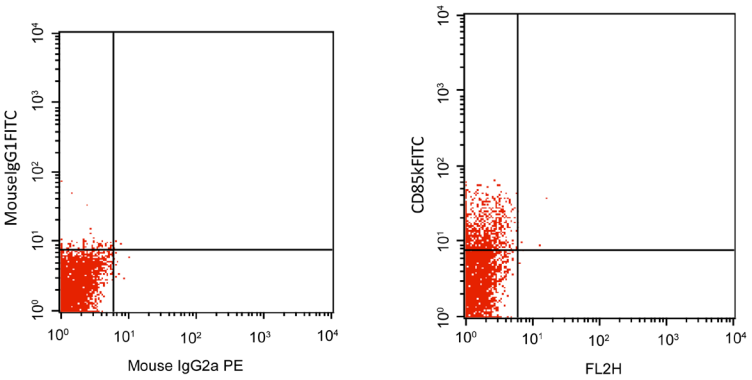
they control cellular transduction, any up or down regulation alters the antigen recognition and promotes leukemic cells spread that is happen by CD85k which is immunoglobulin inhibitory receptor responsible for immune escape. CD36 is the glycoprotein responsible for cellular adhesion and infiltration processes, its overexpression leads to tumor metastasis and organ affection [1] [3] [4] [15] [17]. Formally cytochemical stains can confirm the leukemic cell type, the non-specific esterase was used to identify monocytic component of poorly differentiated monoblastic leukemia [18]. Now with the advance in myeloid and monocytic lineage specific panels, and the IPT become the first and accurate laboratory test [19]. Also characterizations the biological function of each marker facilitate the associated correlation between different cell markers and clinical data [20]. The current study was carried out on thirty two newly diagnosed AML patients according to morphological, cytochemical and IPT criteria. The frequency of CD85k in was 59.37% in AML and 100% in MLIL, CD85k, which has more



Acquisition Date: 06-Apr-16 Gate: G1
Gated Events: 2402 Total Events: 10000
X Parameter: FL2-H (Log) Y Parameter: CD85k FITC (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	551	22.94	5.51	1.54	1.39	22.37	18.32
UR	21	0.87	0.21	6.03	5.79	34.58	28.35
LL	1825	75.98	18.25	1.42	1.33	2.43	2.13
LR	5	0.21	0.05	5.54	5.52	3.86	3.68

Figure 1. FCM analysis of CD85k in AML M4 patient.



Quadrant Statistics

File: Data.002 Log Data Units: Linear Values
Sample ID: PatientID:
Tube:Untitled Panel:Untitled Acquisition Tube List
Acquisition Date: 06-Apr-16 Gate:G1
Gated Events:6004 Total Events:10000
X Parameter:FL2-H(Log) Y Parameter: CD85kFITC(Log) Quad Location: 6,8

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1164	19.39	11.64	1.71	1.52	15.21	13.44
UR	6	0.10	0.06	8.84	8.13	19.13	16.85
LL	4832	80.48	48.32	1.35	1.27	3.03	2.51
LR	2	0.03	0.02	6.71	6.70	6.24	6.13

Figure 2. FCM analysis of CD85k in AML M2 patient.

Table 3. Comparison betweenMLIL and other types of AML as regards IPT.

FCM Markers Expression %	MLIL (No. = 14)	Other AML (No. = 18)	P value
CD45 Mean ± SD	74.70 ± 17.63	89.744 ± 7.0932	0.002 (S)
CD33 Mean ± SD	79.9 ± 12.93	63.40 ± 30.57	0.06 (NS)
CD13 Mean ± SD	58.31 ± 21.41	52.69 ± 28.0343	0.539 (NS)
MPO Mean ± SD	2.302 ± 2.27	6.737 ± 21.56	0.451 (NS)
CD38 Mean ± SD	36.88 ± 36.34	67.32 ± 26.79	0.01 (S)
CD34 Mean ± SD	0.760 ± 0.460	26.15 ± 31.58	0.005 (S)
CD 117 Mean ± SD	1.98 ± 1.843	25.24 ± 31.951	0.011(S)
HLADR	69.321 ± 23.642	37.989 ± 31.379	<0.001 (HS)
CD15 Mean ± SD	31.731 ± 26.567	4.972 ± 3.808	<0.001 (HS)
CD14 Mean ± SD	38.421 ± 39.79	5.47 ± 4.43	<0.001 HS
CD 36 Mean ± SD	0.9800 ± 1.004	15.69 ± 23.616	0.027 (S)
235a Mean ± SD	0.7143 ± 0.3697	17.798 ± 16.831	<0.001 (HS)
CD 61 Mean ± SD	2.881 ± 1.749	8.308 ± 7.216	0.01 (S)
CD85K Mean ± SD	43.235 ± 19.465	14.232 ± 22.070	<0.001 (HS)

frequent association with MLIL but not specific marker for MLIL, because it also expressed on other cell lineage as lymphatic as mentioned by Xunlei (2016) [10]. CD85k is more frequent at age of below 35 years old without sex difference, and showed only significant value to TLC more than 50,000, and Hb level less than 7 g/dl, non-significant values with other hematological variables and organomegaly, this in relevant to the fact that its expression is indicative for biological origin of the MLIL and has prognostic value rather than tumor burden. These data are in agreeing with Kang *et al.* (2015) [16]. We noticed that; CD85k despite its positive frequency with MLIL, it is insignificantly correlate with other monocytic markers including CD36, CD14, CD15, but significant correlation with progenitor markers CD34, HLA-DR, may be originate to the biological role of each one

differs in leukemia development, CD85k has a role in clonality association and immune escape, other results were correlated with clinical features, peripheral blood lymphocytosis, platelets count and hemoglobin level [21]. Also our results was consistent with Dobrowolska *et al.* (2013); who reported that there were no significant differences between CD85k positive or negative groups for the following parameters: age, sex, WBC, hemoglobin, and platelet count. In their work, they revealed that; the inhibitory receptor CD85k was sensitive and specific marker for MLIL diagnosis and follow up [22]. In this study there is no correlation between CD85K and P.Bl variables as WBCs less than 50,000 and Hb more than 7 g/dl or platelet count in AML group and control group, while Cagnetta *et al.* (2014), in their study showed that the expression of CD 85K was related to age, WBC counts, and Platelet counts in P.Bl respectively ($p < 0.05$), CD85k was poorly associated with of hemoglobin level [23]. Our study found that there was positive significant correlation between CD85K and each of HLA-DR, negative significant correlation between CD85K and each of CD16, CD117, CD36, and no correlation with other types of IPT. When we made comparison between (M4, M5) and other types of AML, we found that there was significant statistical value of CD45, CD14, CD15, CD34, CD38, CD36, CD16, CD85K, HLADR, 235a. Dobrowolska *et al.* (2013), found in their research that CD85k was co-expressed by positive CD34/negative (CD117/CD14), M4/M5, also by more differentiated (negative CD34, CD117, CD14) leukemic cells. Overall, the co-expression with CD117 was presented 50%, while co-expression with CD34 was seen in 39% of MLIL [22]. According to Zhang (2015) and Petz *et al.* (2015); whom found that; the co-expression of CD85k and progenitor markers may be interpreted as asynchronous proliferation of leukemic cells [9] [24]. Thus, our findings are in accordance of Hao Cheng *et al.* (2011) and Cagnetta *et al.* (2014), whom reported that segregation of myeloid and monocytic precursors occurs at an early step of hematopoietic differentiation [1] [23]. Our results demonstrated that; the expression of ILT3 is absent in the control group, ILT3 is expressed in 19 patients in AML group (59.38%), ILT3 was expressed by 14/14 cases of AML (M4/M5). This is in agreement with Dobrowolska *et al.* (2013) that show in their study that the inhibitory receptor ILT3 is a highly sensitive and specific marker for the diagnosis and monitoring of AML with monocytic differentiation, ILT3 was expressed by all cases of AML with monocytic differentiation and in none of the AML cases, which included M1/M2 and M3. The distinction between monocytic AML and other AML types is extremely important particularly in the differential diagnosis of AML with monocytic differentiation which requires different treatment strategies [22]. It is essential for the generation of regulatory T cells in humans; up regulation of this inhibitory receptor plays a crucial role in graft adaptation and protection against the recipient's immune response as reported by Xunlei *et al.* (2016) [10] [25].

4. Conclusion

These findings give the potential value of CD85k application in MLIL therapy.

By the use of specific CD85k Mo Abs rendering leukemic cells more susceptible to anti-tumor T cell regulation. In practice it has no role in diagnosis or clinical assessment of the disease, but it's a main role in prediction after chemotherapy, so it is a good marker for follow up. In patients with ITP (control), CD85k has no biological role in pathophysiology of the disease. Here we find that in leukemia, CD85k expression is up regulated the reverse occurred in ITP, down regulation of CD85k, the usefulness of these data is that; some experimental manipulations in cellular signal transduction of ILT-3 in these diseases by downregulation in leukemia or upregulation in ITP can alter the fate of the disease. And be useful in therapy.

Limitations of This Study

The small sample size of the patients, most patients were died before complete the follow up.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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