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ORIGINAL ARTICLE

Study of CD160 expression in B-chronic Lymphocytic Leukemia.

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ABSTRACT

Background: B-cell lymphoproliferative disorders (B-CLPD) are a biologically heterogeneous group of malignant diseases characterized by accumulation of mature B lymphocytes in the bone marrow, peripheral blood and lymphoid tissues. B-CLPD is now mostly diagnosed by flow cytometric immunophenotyping

Aim of work: to throw light on the expression of CD160 in B – chronic lymphocytic leukemia and demonstrate if it can differentiate B – CLL from other causes of absolute lymphocytosis and to correlate its expression with laboratory findings and immunophenotyping .

Subjects and methods: The study included 20 patients recently diagnosed as chronic lymphocytic leukemia who attended medical oncology department of Zagazig University hospitals between April 2017 and April 2018 and 20 cases with other causes of absolute lymphocytosis from outpatient clinic. All studied patients were subjected to full history taking, complete clinical examination and laboratory investigations including CBC Leishman - stained peripheral blood smears for differential leucocytic count, liver functions, kidney functions, serum LDH level, Hepatitis C virus antibody, Hepatitis B surface antigen, HIV Ag, Ab and immunophenotyping. Measuring expression of CD160 on B cell lymphocytes in combination with CD19 in both patients and control groups.

Results: There were no significant correlations between CD160 and hematological findings, LDH, liver and kidney function findings. There was significant positive correlation between CD160% and CD23% and CD5%, while there was no significant correlation between CD160 and other flow markers expression in CLL.

Conclusion: CD160 is expressed on a greater percentage of B – CLL cells in patients than in control group with very high sensitivity and specificity. CD160 represents a potential and additional diagnostic marker of CLL cases.

Keywords: B-chronic Lymphocytic Leukemia, CD160.

INTRODUCTION

B-cell lymphoproliferative disorders (B-CLPD) are a biologically heterogeneous group of malignant diseases characterized by accumulation of mature B lymphocytes in the bone marrow (BM), peripheral blood, and lymphoid tissues. **[1]**
B-CLPD is now most often diagnosed by flow cytometric immunophenotyping that identifies a clonal light-chain restricted population expressing B-cell markers in the blood or BM. **[2]**
CLL classically displaying a score of 4 or 5 according to the widely used flow cytometry score developed by Estella Matutes. **[3]**
However, some CLL cases may have an atypical immunophenotype (e.g., CD5-, CD23- or FMC7+) and/or morphological features resulting in diagnostic uncertainty. Furthermore, CLL and other B-cell neoplasms in leukemic phase, especially CD5+ B-cell lymphocytosis mainly mantle cell lymphoma can share close morphologic features increasing diagnostic confusion. **[4]**

CD160 is a glycosylphosphatidylinositol-anchored cell surface molecule belonging to the immunoglobulin super family (IgSF). It is a 27 kDa glycoprotein that is known as an essential natural killer (NK) cell activating receptor. Its expression is associated tightly with peripheral blood NK cells and CD3+ CD8+ T lymphocytes with cytolytic effector activity. Some reports have shown that CD160 is expressed by CLL cells, but not in normal B lymphocytes. **[5]**

It has also been demonstrated that CLL cells can be regulated by the CD160 pathway for in vitro proliferation and activation. Further, activated CD160 protects CLL cells from rapid spontaneous apoptosis in vitro via the phosphatidylinositol 3-kinase (PI3K)/AKT pathway. **[6]**

Aim and objectives: to throw light on the expression of CD160 in B – chronic lymphocytic leukemia and demonstrate if it can differentiate B – CLL from other causes of absolute lymphocytosis and to correlate its expression with laboratory findings and immunophenotyping.

SUBJECTS AND METHODS

Technical design:

Study design: The present study was conducted on 20 patients recently diagnosed as chronic lymphocytic leukemia who attended medical oncology department of Zagazig University hospitals between April 2017 and April 2018 and 20 cases with other causes of absolute lymphocytosis from outpatient clinic.

Studied groups: Group I included 20 patients who were diagnosed as B- chronic lymphocytic leukemia based on clinical, hematological and immunophenotypic criteria. They were 13 males (65%) and 7 females (35%). Their age ranged between 47-75 years. Group II included 10 cases with benign lymphocytosis, they were 5 (50%) males and 5 females (50%), there age ranged between 22-45 years. Group III included 10 patients with B-NHL. They were 3 males (30%) and 7 females (70%). Their age ranged between 35-71 years.

Inclusion criteria: All cases diagnosed as CLL at different stages and patients with absolute lymphocytosis.

Exclusion criteria: Other non-hematological malignancies and other immunological disorders diagnosed clinically and laboratory.

Methods: All studied patients were subjected to Complete history taking, laying stress on history of fever, weight loss and night sweats. Also, Clinical examination, laying stress on lymphadenopathy, splenomegaly and hepatomegaly was done. Laboratory investigations were done including: Complete blood picture (CBC) using automated cell counter “Sysmex XN, Japan”, together with examination of Leishman - stained peripheral blood (PB) smears for differential leucocytic count with laying stress on lymphocyte percentage and morphology, Blood chemistry including Liver functions, kidney functions and serum LDH level using automated analyzer “cobas 8000 platform – 702c module, Germany”, Hepatitis C virus antibody (HCV Ab), Hepatitis B surface antigen (HBsAg) and HIV Ag, Ab using automated analyzer “cobas 8000 platform – e602 module, Germany”, Immunophenotyping using (FACScalibur, Becton Dickinson, San Jose, California, USA) flow cytometer. Acquisition and analysis were performed using CellQuest software (BD Biosciences) **[7]**. The panel of fluorescin isothiocyanate (FITC), phycoerythrin (PE) and peridinin -chlorophyll-protein (PerCP) conjugated monoclonal antibodies were used for the diagnosis which included: CD5, CD19, CD20, CD23, CD79b, FMC7, CD10, CD38, Kappa and Lambda. Matutes scoring system was used to diagnose cases of CLL and lymphoma. Cases considered typical for CLL have scores of 4 or 5 (15 cases). Atypical CLL cases have scores of 3 or less (5 cases) .Scores of 0-2 are considered other B-cell leukemia and NHL (10 cases). **[8].**Measuring expression of CD160 on B cell lymphocytes in combination with CD19 in both patients and control groups.

Administrative considerations: Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University (Institutional Research Board IRB). The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis: Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20, USA. Quantitative variables were described using their means,medians and standard deviations. Nonparametric test (Mann Whitney) was used to compare means when data was not normally distributed and to compare medians in categorical data. To compare means of more than two groups, one-way ANOVA was used for normally distributed data and Kruskal Wallis test was used for data which wasn’t normally distributed. ROC curve analysis was used to assess the best cutoff of studied parameters. The level statistical significance was set at 5% ($P<0.05$). Highly significant difference was present if $p\leq0.001$.

RESULTS

Table (1) Show the percentage of CD160 of the studied groups.

Table (2) Shows Mini- CLL score for CLL and B-NHL groups which consists of the 3 most expressed markers in CLL (CD5,CD23 and CD160) with a score of 3 differentiate CLL from B-NHL and a score of 0 exclude CLL.**(4)**
Table (3) Shows the percentage of CD160 in both typical & atypical CLL cases.
Table (4) Shows the relation between CD160 and clinical findings in CLL cases.
Table (5) Shows the correlation between CD160 in CLL cases and CBC findings.
Table (6) shows the correlation between CD160 and other flow markers expression in CLL cases.
Table (7) Shows the CD160 MFI of the studied groups.

Table (1): CD160% of studied groups

CD160%	Group I CLL (20 cases)	Group II Benign lymphocytosis (10 cases)	Group III B-NHL (10 cases)	KW Test	p	
Mean ± SD	62 ± 14.88$^{\infty}$	5.66 ± 4.4	1.9 ± 2.42	29.171	<0.001 (HS)	
Median	61.45	7.01	0.3			P1 <0.001
Range	31.98 – 87.72	0.04 – 13.35	0.02 – 5.71			P2 <0.001
						P3 0.777

P<0.05 is significant
P1 indicates p value of test of significance between Group I and Group II
P2 indicates p value of test of significance between Group I and Group III
P3 indicates p value of test of significance between Group II and Group III
It shows that there was high significant difference between the three groups regarding CD160 where it was positively expressed in all cases of CLL and negatively expressd in all cases of the other 2 groups.

Table (2): Mini- CLL score for CLL and B-NHL groups

	CD5	CD23	CD160	Mini–CLL Score
CLL group (N=20)	+	+	+	**3**
NHL group (N=10)	+	–	–	**1**

All 20 cases of CLL group had a mini – score of 3, while in B-NHL group the 10 cases had a score of 1.

Table (3): CD160 % in typical & atypical CLL

CD160%	Typical CLL (15 cases)	Atypical CLL (5 cases)	KW test	P
Mean ± SD	62.5 ± 15.26	62.9 ± 13.6	27.311	0.703
median	63.86	57.31		
Range	31.98 – 87.72	48.5 – 81.58		

KW is Kruskal Wallis test
P<0.05 is significant
There was no significant difference between CD160 % in both typical & atypical CLL cases, so it could help to differentiate between atypical B-CLL & B-NHL.

Table (4): Relation between CD160 % and clinical findings in studied cases of CLL:

CLL case group	CD160 %	T	p	significance
	Mean ± SD			
Lymphadenopathy:				
Absent	47.93 ± 11.23	-0.967	0.346	NS
Present	62.72 ± 14.9			
Splenomegaly:				
Absent	67.66 ± 17.37	0.847	0.408	NS
Present	60.56 ± 14.47			
Hepatomegaly:				
Absent	65.73 ± 15.17	0.729	0.475	NS
Present	60.37 ± 15.03			

There were no significant differences between clinical examination findings and CD160 %.

Table (5): Correlation between CD160 % in CLL patients and CBC findings:

CLL case group		r	p	Significance
CD160%	**TLC**	0.382	0.096	NS
	Lymphocytes%	-0.136	0.569	NS
	ALC	0.387	0.092	NS
	HB	0.213	0.368	NS
	Platelet	-0.122	0.608	NS
	Reticulocytes	-0.009	0.970	NS

R is Spearman correlation
P<0.05 is significant
There were no significant correlations between CD160% and hematological findings.

Table (6): Correlation between CD160% and other flow markers expression in CLL patients:

CLL case group		R	p	Significance
CD160%	**CD5/CD20%**	0.303	0.194	NS
	CD23%	0.508	0.022	S
	CD79b%	-0.085	0.722	NS
	FMC7%	0.147	0.537	NS
	CD38%	0.023	0.922	NS
	CD10%	0.279	0.233	NS
	CD19%	0.315	0.177	NS
	CD5%	0.451	0.012	S

R is Spearman correlation
P<0.05 is significant
There was significant positive correlation between CD160% and CD23% and CD5%, while there was no significant correlation between CD160 and other flow markers expression in CLL.

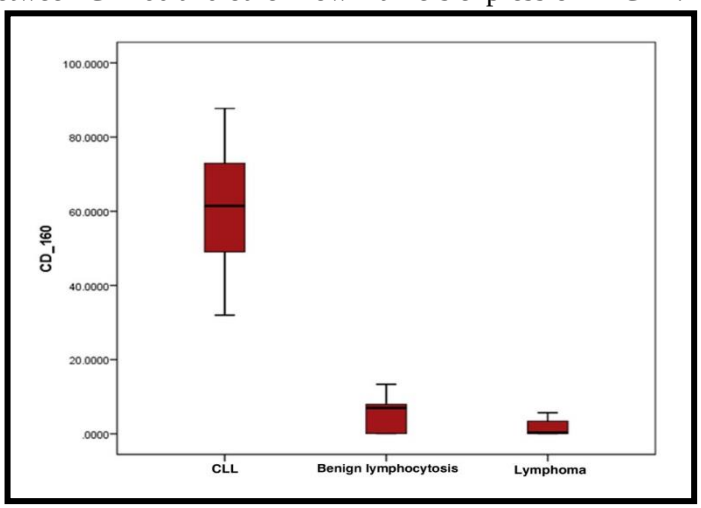

Figure (1): Boxplot showing CD160 levels in studied groups

DISCUSSION

B-cell chronic lymphocytic leukemia is a heterogeneous disease with a highly variable clinical course and prognosis. Clinical staging of CLL and determination of the extent of the disease are conducted with the Rai staging system or Binet classification. The two major clinical staging systems are unable to discriminate prospectively between an indolent or aggressive course within the low and intermediate risk categories. For this reason, some biological parameters have been used for the CLL staging systems to differentiate subsets of prognostics. **[9]**

It has been demonstrated that CLL cells can be regulated by the CD160 pathway for in vitro proliferation and activation. Further, activated CD160 protects CLL cells from rapid spontaneous apoptosis in vitro via the phosphatidylinositol 3-kinase (PI3K)/AKT pathway. Thus, we hypothesized that CD160 may be highly expressed in CLL cells. **[6]**
To highlight the relevance of CD160 expression in B-CLL, our study was conducted on the clinical significance of CD160 expression and if it can differentiate B-CLL from other causes of lymphocytosis.

As regard CD5/CD20 coexpression , it was positive in all cases (100%) of both CLL and B-NHL groups with presence of high significant level in CLL group where it ranged from (29.6 to 92.9 %), while it was negatively expressed in all cases of benign lymphocytosis group. In the group of B-NHL beside the positivity of CD5/CD20, cycline-D was done for those patients from bone marrow biopsies. It was positive in six (6) cases and diagnosed Mantle cell lymphoma and negative in four (4) cases who diagnosed as diffuse large B cell lymphoma.

As regards CD23 expression, it was positive in 100% of CLL cases. Its positivity was diagnostic for CLL ($P<0.001$), while it was negative in all cases (100%) of both B-NHL and benign lymphocytosis groups. Regarding CD5 and CD19, they were positively expressed in 100% of all cases of both CLL and B-NHL groups with presence of high significant difference between CLL and NHL regarding CD5.

In benign lymphocytosis group, CD19 was positive in 2 cases (20%) and negatively expressed in the remaining cases, while CD5 was positive in 80% of cases with presence of high significant difference between that group and CLL group regarding CD5.

As regard to CD10, it was negatively expressed in all cases of the 3 groups and there was no case of follicular lymphoma detected in our study. CD38 is considered as one of the prognostic markers in CLL patients, in our study, it was expressed in (5/20) of CLL cases (25%).

In work similar to our findings, Abdel-Ghafar and colleagues reported in their study 35 adult patients newly diagnosed with B cell chronic lymphoproliferative neoplasm's (B-CLPN). They were classified as 22 cases diagnosed with non-Hodgkin lymphoma (NHL) and 11 cases diagnosed with chronic lymphocytic leukemia (CLL) and 2 cases with hairy cell leukemia (HCL). By immunophenotypic analysis of B lymphocytes, all cases in CLL group expressed CD19, CD20, CD23 and CD5, 36% expressed CD38 and 68.0% were negative for CD79b. In the second group, NHL group, lymphocytes showed to express pan B-lymphocyte markers, CD20 (100% with bright intensity), CD19 (100%) and CD79b (86.0%, bright expression). Finally, CD10 was expressed in one case. **[10]**

As regard CD160, CLL group had the significant higher level than the other two groups, it was expressed in 100% of our cases. The median percent expression was 61.45% (range 31.98 - 87.72%) and median fluorescence intensity (MFI) 34.55 (range 23.1 - 45.4). In B-NHL group, the median percent expression was 0.3% (range 0.02-5.71%) and MFI 2.6 (range 1.1-5.2) ($p < 0.001$) and this was consistent with Farren, Zhang and colleagues who stated that CD160 was expressed specifically in all CLL patients and may be also a potential diagnostic marker. **[4],[11]**

Also, in our study CD160 proved to be completely negative on B lymphocytes of benign lymphocytosis group and these results were similar to those reported by Lesesve and colleagues. **[12]**

In our study we found that the percent of CD160 positive cells in atypical CLL samples were similar to the typical cases. Its range was from (48.5 to 81.58 %) with median of 57.31 % in atypical CLL and from (31.98 to 87.72 %) with median of 63.86 % in typical ones with no significant difference between them.

Also, Liu and colleagues examined expression of CD160 in 54 CLL patients, the positivity was seen in 53 patients and in their study they demonstrated that CD160 promotes CLL survival by up regulation of BCL2, BCLx and MCL expression and blocking cytochrome c release and caspase activation. **[6]**

As regard to our study, we detected that there were no significant correlations between CD160 and hematological findings, LDH, liver and kidney function findings. Considering immunophenotyping markers, there was no significant correlation between CD160 versus CD5/CD20, CD19, CD79b, FMC7, CD38 and CD10, but there was significant positive correlation between CD160 and both CD23 and CD5.

Similar to our results, Lesesve and colleagues reported that no statistically significant correlation was observed between CD160 and absolute lymphocytic count (ALC) and CD38 flow marker, where 20 of 69 patients with CLL included in their study were positive for CD38 but no statistically significant correlation was observed between CD38 and CD160 cell surface expressions. **[12]**

In contrast to our results, Zhang and colleagues in their study which was performed on 57 patients with CLL, they observed that there was significant positive correlation between CD160 and high total leucocytic count (TLC), absolute lymphocytic count (ALC), CD19+ and CD20+ flowcytometry markers, except for the positive correlation between CD160 and CD5+ and CD23+ , they were only similar to our result and the difference may be due to large sample size. **[11]**

Our data confirm that CD160 cell surface expression was absent on normal B-lymphocytes, expressed in 100% of B-CLL and also, absent on all cases of B-NHL. This emphasized the utility of CD160 in the differential diagnosis of B-cell lymphoproliferative neoplasms with ambiguous features.

CONCLUSION

CD160 is expressed on a greater percentage of B – CLL cells in patients than in control group with very high sensitivity and specificity, so it can represent a potential and additional diagnostic marker of CLL cases and may be included in a multiparameter approach as a second step in ambiguous CLL cases. Also, CD160 is expressed specifically on B-CLL cells and this correlation suggests that CD160 monoclonal antibody may well become a new method of treatment for CLL. CD160 is also a sensitive and specific marker for discriminating between CLL and MCL in flow cytometry immunophenotypic analysis and is particularly useful in recognizing atypical CLL. CD160 expression may be a useful marker to differentiate atypical CLL from other B-cell neoplasms in the absence of available biopsies or cytogenetics and molecular studies.

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