Original Article

Expressions of immunoglobulin M and immunoglobulin D heavy chain in B-chronic lymphocytic leukemia and their correlation with CD38 expression

Ghid Nahd Abdul Muhssen, Hind Shaker AL-Mamoori, Majid Hameed Ahmed[1]

Abstract:

BACKGROUND: B-chronic lymphocytic leukemia (CLL) is a monoclonal malignancy characterized by an accumulation of small mature-looking B lymphocytes in the blood, bone marrow, and other tissues. It is typically characterized by CD5+, CD23+, CD22−, and CD79b−, with weak expression of surface immunoglobulin (Ig).

OBJECTIVES: The aim of the study was to assess the expression of IgM and IgD heavy chain by flow cytometry in untreated newly diagnosed B-cell CLL and correlate the heavy chain isotopes expression with CD38 expression.

MATERIALS AND METHODS: A prospective cross-sectional study was conducted on thirty patients with new diagnosis of B-CLL. The study was conducted at nursing home teaching laboratory in medical city hospital in Baghdad for the period from November 2016 to March 2017. Five milliliter of venous blood in ethylenediaminetetraacetic acid was collected from thirty patients for detection of expression of IgM, IgD, and CD38 by flow cytometry. The patients were randomly selected regarding the age, sex, and stage of the disease.

RESULTS: The mean age of all included patients was (61.73 ± 6.92) with more disease predominant in male than female. The most common presenting feature of the patients was lymphadenopathy found in 16 patients (43%). Regarding staging system, six (20%) patients were in with Binet Stage C and five patients (17%) were in modified Rai Stage 4. CD38 and IgM expression showed a significant relation to hemoglobin and platelets ($P < 0.05$). There was a significant positive correlation between IgM and CD38 while it was negative between IgD and CD38.

CONCLUSIONS: The current study showed that the expression of CD38 had a significant correlation with IgM expression as well as there was positive correlation with the stage of the disease which may point out to inferior prognosis for those patients with CLL.

Keywords:

CD38, chronic lymphocytic leukemia, immunoglobulin heavy chains

Introduction

Chronic lymphocytic leukemia (CLL) is the most common lymphoid malignancy in the Western world, but its incidence is low in the Far East and characterized by the accumulation of mature clonal of CD5+ B-cells.[1] The disease characterized by its clinical heterogeneity, in which some patients progressing rapidly with early death and others exhibit a more stable, slow-growing disease with minimal changes in blood cell count lasting many years. Hence, it is important to develop sensitive stratification parameters to identify patients with poor prognosis. The incidence of CLL ranges from 1 to 5.5/100,000 people in worldwide with median age of 72 years and more common in more common in western

Departments of Pathology and [1]Physiology, College of Medicine Al-Nahrain University, Baghdad, Iraq

Address for correspondence:
Dr. Ghid Nahd Abdul Muhssen,
Department of Pathology,
College of Medicine
Al-Nahrain University,
Baghdad, Iraq.
E-mail: ghidnahd@
yahoo.com

Submission: 31-05-2017
Accepted: 13-08-2017

countries than others and in males than in females.[2] Two major clinical staging systems (Rai and Binet staging), mainly based on tumor load, were developed to estimate prognosis in CLL.[3] Many studies concentrated on the identification of surrogate markers with similar prognostic value, and whose expression could be easily verifying by flow cytometry; for example, expression of CD38, expression of immunoglobulin M (IgM), and IgD heavy chain in CLL have been shown to correlate with unfavorable prognosis.[4]

CD38 appears to be a global molecular bridge to the environment, promoting survival/proliferation over apoptosis. Furthermore, this evidence contributes to the current view of CLL as a chronic disease, in which the host's microenvironment promotes leukemic cell growth and also controls the sequential acquisition and accumulation of genetic alterations.[5]

In CLL, the relationship between CD38 expression and Ig heavy chain expression is indirectly reflecting normal maturation. Thus, it has a pivotal role in initiating and modulating a series of input signals from the microenvironment.[6] In the mantle zone of normal human lymphoid follicles, the naive IgM/IgD-bearing B-cells without CD38 in the germinal center may become IgM+ memory cells and/or isotype (class-switching), which could become IgG- or IgA-bearing cell. While the postgerminal center memory B-cells are heterogeneous with respect to heavy chain expression that including IgM+/IgD+, IgM-only, and class-switched cells that lack expression of CD38. A subset of germinal center and peripheral blood B-cells expressing IgD without IgM has been identified. These memory B-cells are remarkable for a relatively high load of Ig heavy chain variable region (IGHV) gene mutation.[7]

Because normal CD5+ B-cells are present in the mantle zone of lymphoid follicles, B-cell CLL is most likely a malignancy of a mantle zone. Although B-CLL had been regarded as a malignant neoplasm of pregerminal center B-cells, some significant proportion have undergone somatic mutation of IGHV genes implying that these cells have transited the germinal center.[8] This study was undertaken to find the importance of such markers to detect the possible correlation of these markers with the disease prognosis of CLL patients.

Materials and Methods

A prospective cross-sectional study was conducted on thirty patients with new diagnosis of B-CLL. The study was conducted at nursing home teaching laboratory in medical city hospital in Baghdad for the period from November 2016 to March 2017. Five milliliter of venous blood in ethylenediaminetetraacetic acid was collected from thirty patients for detection of expression of IgM, IgD, and CD38 by flow cytometry. The patients were randomly selected regarding the age, sex, and stage of the disease.

The panel that was used for diagnosis of CLL was SmIg, CD5, CD23, FMC-7, and CD79b that typically show score (4–5) according the FCM scoring system for diagnosis CLL.[9] A written informed consent obtained from all patients before study. The study was approved by the review ethical committee at Al-Nahrain University College of Medicine.

Flow cytometric immunophenotyping

Four-color immunophenotyping data were attained using a FACSCalibur flow cytometer (Partec Cyflow® Cube 6, Germany). Fluorochrome-conjugated monoclonal antibodies that usually used as follows: CD3; CD5; CD10; CD19; CD20; CD23; CD45 and FMC-7; CD38, and IgM, and IgD. List mode files were analyzed using Cell Quest software (BD Biosciences). Atypical immunophenotypic characteristics in B-CLL were bright CD20 fluorescence, bright surface Ig light chain fluorescence, or positivity for FMC-7, and the positivity for CD38 was determined by comparison with the isotype control, and the CD38 + percentage cells among gated CD19+/CD5 + cells were calculated. The antigen expression (CD38) was considered to be positive when the percentage of positive cells was equal or >7%.[9] The statistical analysis of this cross-sectional study performed with the IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. and Microsoft Excel program Ver. 3. 2013, Chicago. Numerical data were described as mean and standard error. Analysis of variance was used for comparison among three groups. While, categorical data described as count, percentages, and Chi-square test used to estimate the association between variables. For the tables with frequencies, range, mean, and standard deviation values were considered statistically significant difference when $P < 0.05$.

Results

Thirty adult newly diagnosed CLL patients were enrolled in this study; the mean age was 61.73 ± 6.92 years (mean ± standard deviation [SD]); the highest incidence was reported at the age group >50 years 96%. CLL was observed more in males than in females with Male: female ratio of 3:2, and the most common presenting feature of CLL patients was lymphadenopathy (16 cases) 53%. Six (20%) of patients were within Binet Stage C and 5 (17%) of patients were within Rai Stage 4 as shown in Table 1.

Markers expression

The percentage of positive expression IgM was detected in 9/30 patients (30%) while IgD expression that detected

in 17/30 patients was (57%), and CD38 expression in 8/30 patients was detected (27%) as shown in Table 2.

The result showed that there were highly significant differences in expression of CD38 and IgM in Binet stages ($P < 0.001$) as all six cases of Binet Stage C showed positive expression of CD38 and IgM in comparison to Binet Stage A which showed only one case-positive expression for IgM from 20 cases and nil for CD38 expression. Regarding expression of IgD, also there was a significant difference in Binet stages ($P = 0.029$) as the expression was 100% in Binet Stage B, 60% in Binet Stage A, and only 16.7% in Binet Stage C as demonstrated in Table 3.

The result showed that there were highly significant differences in percentage of expression of IgM in Rai stages with ($P < 0.001$) as all cases in high stage (100%) showed positive expression IgM in comparison to 0.0% and 28.7% in low and intermediate stages, respectively. The expression of CD38 was (100%) in high stage in comparison to 0.0% and 21.4% in low and intermediate stages, respectively. The positive expression of IgD was significantly different in Rai stages (P value = 0.049) as it was positively expressed in 11 of 14 cases of intermediate stage in comparison to 5 of 11 in low stage and 1 of 5 in high stage as seen in Table 4.

In this study, only five CLL cases showed positive expression for CD38 and IgM isotopes expression, two cases had positive expression for CD38 and IgD isotopes expression, yet one case only showed positive expression for all studies, three markers as demonstrated in Table 5.

Table 1: Demographic, clinical, and laboratory parameters of chronic lymphocytic leukemia patients

Parameter	Frequency (%)
Age (years)	
>50	29 (96.67)
<50	1 (3.33)
Mean±SD (range), years	61.73±6.92 (45-73)
Gender	
Female	12 (40)
Male	18 (60)
Male:female ratio	3:2
Clinical features number of patients (%)	
Lymphadenopathy	16 (53.3)
Fever	15 (50)
Splenomegaly	12 (40)
Pallor	9 (30)
Hepatomegaly	9 (30)
Fatigue	1 (3.3)
Hematological parameters mean±SD (range)	
WBC (×10⁹/L)	51.08±44.73 (10.7-206)
Lymphocyte (×10⁹/L)	43.38±42.25 (6-185)
Hemoglobin (g/dl)	11.49±2.52 (6.1-15.5)
Platelets (×10⁹/L)	177.64±75.12 (50-391)
Staging system number of patient (%)	
Binet staging system	
A	20 (67)
B	4 (13)
C	6 (20)
Modified Rai staging system	
Low	11 (36)
Intermediate 1	8 (27)
Intermediate 2	6 (20)
High	5 (17)

SD=Standard deviation, WBC=White blood cell

Table 2: Surface markers immunoglobulin M, immunoglobulin D, and CD38 expression in chronic lymphocytic leukemia cases

Marker	Frequency (%)
IgM	
Positive	9 (30)
Negative	21 (70)
IgD	
Positive	17 (57)
Negative	13 (43)
CD38	
Positive	8 (27)
Negative	22 (73)

IgM=Immunoglobulin M, IgD=Immunoglobulin D

Table 3: Markers expression percent in relation to Binet staging

Parameter	Binet			P
	A, n (%)	B, n (%)	C, n (%)	
IgM				
Positive	1 (5.0)	2 (50.0)	6 (100)	<0.001
Negative	19 (95.0)	2 (50.0)	0	
IgD				
Positive	12 (60.0)	4 (100)	1 (83.3)	<0.029
Negative	8 (40.0)	0	5 (16.7)	
CD38				
Positive	0	2 (50.0)	6 (100)	<0.001
Negative	20 (100)	2 (50.0)	0	

IgM=Immunoglobulin M, IgD=Immunoglobulin D

Table 4: Immunoglobulin M, immunoglobulin D, and CD38 in relation to Rai staging

Parameter	Modified Rai stages			P
	Low, n (%)	Intermediate, n (%)	High, n (%)	
IgM				
Positive	0	4 (28.6)	5 (100)	<0.001
Negative	11 (100)	10 (71.4)	0	
IgD				
Positive	5 (45.5)	11 (78.6)	1 (20.0)	0.049
Negative	6 (54.5)	3 (21.4)	4 (80.0)	
CD38				
Positive	0	3 (21.4)	5 (100)	<0.001
Negative	11 (100)	11 (78.6)	0	

IgM=Immunoglobulin M, IgD=Immunoglobulin D

Table 5: Relation of positive CD38 expression with other markers immunoglobulin M, immunoglobulin D isotopes expression

CD38	IgM positive	IgD positive	IgM and IgD positive	IgM and IgD negative	Total
Positive	5	2	1	0	8
Negative	0	11	3	8	22

IgM=Immunoglobulin M, IgD=Immunoglobulin D

Discussion

Mature B-CLL is a B-lineage lymphoid malignancy, characterized by absolute lymphocytosis in the peripheral blood and bone marrow.[1]

In this study, the mean age of all patients included was 61.73 ± 6.92 SD, with a median of 61.73 years old and a range of (45–73) years old. The result was similar to other national and international studies.[10,11] Regarding the clinical and laboratory characteristic, the results of this study were comparable to that published by Jasim Mohammed *et al.*, Jasim *et al.*, and Karen *et al.*.[10,12,13]

This study showed that 67% of patients were in Binet Stage A and 63% in modified Rai including low and intermediate 1, which reflect that most patients were in early stage, this result was similar to study by Cantu *et al.*[14] which revealed higher percentage of patients within Stage A reaching up yo 60%; In contrast Jaafar *et al.*[15] had found that most CLL patients were within Binet Stage C (63.3%), this can be attributed to an early diagnosis of cases and routine checkup of our patients.

The current study showed that there was highly significant correlation between IgM isotope and CD38 expression with Binet staging system of mature B-CLL; in which it was found that only one case within Binet Stage A (5%), two cases within Binet Stage B (50%), and six cases within Binet Stage C (100%) that showed positive expression of IgM isotope ($P < 0.001$); while number of negative cases were 19 cases found within Binet Stage A (95%) and 2 cases within Binet Stage B (50%), similar highly significant correlation ($P < 0.001$) was found between CD38 expression and Binet stages. In the same line IgD isotope expression also had significant correlation ($P < 0.029$), where 12 cases were in Binet Stage A (60%), 4 cases in Binet Stage B (100), and finally one case in Binet Stage C (16.7%). Moreover, the negative IgD isotope expression was found in five cases in the Binet Stage A (40%) and five cases in the Binet Stage C (83.3%). These results were in agreement with results of other researchers like Thunberg *et al.*,[16] Shen *et al.*,[17] who showed that CD38 expression associated with IgM isotope expression which highlights that CD38+ displaying a greater disease activity and had been described to play a complex role in the lymphocyte

proliferation. Hence, it could be helpful to detect disease progression in low-risk mature B-CLL patients that have high IgM isotope and CD38 expression at the time of diagnosis.[18]

Rai staging system of mature B-CLL, there was a highly significant correlation ($P < 0.001$) in which four cases were in intermediate-risk group (28.6%) and five cases within high-risk group (100%), whereas the negative IgM isotope expression found in 11 cases within low-risk group (100%),and 10 cases in intermediate-risk group (71.4%). similar high significance was found between CD38 expression and modified Rai staging system ($P < 0.001$), in which 3 positive cases (21.4%) were in the intermediate-risk group and five cases in the high-risk group (100%). However, the negative CD38 expression was seen in 11 cases (100%) in low risk group and 11 cases (78.6%) in intermediate-risk group.

On the other hand, IgD isotope expression had weak correlation with modified Rai staging system with just significant ($P < 0.049$), as there was no difference between positive and negative cases in Low risk group in comparison to intermediate and high risk group. These above results were in agreement with studies by Thunberg *et al.*,[10] Ker *et al.*,[19] and Matrai *et al.*[20] who showed that CD38 expression associated with IgM isotope expression which may refer to possibility of CD38+ positivity in disease progression.

Regarding the relationship between CD38 and IgM, IgD isotopes expressions, among 30 newly diagnosed mature B-CLL cases that included in this study, only 5 cases showed positive expression for CD38 and IgM isotopes expression, 2 cases had positive expression for CD38 and IgD isotopes expression, and only one case showed positive expression for all 3 marker which differ from that reported by Shen *et al.*[17] who stated that percent of CD38+ cells was lowest in IgD negative cases (2.7%) and highest in IgM+/IgD+ cases (75.4%) which may be due to small sample size.

Conclusions

The current study showed that the expression of CD38 had a significant correlation with IgM expression as well as there was positive correlation with the stage of the disease which may point out to inferior prognosis for those patients with CLL.

Financial support and sponsorship

Conflicts of interest
There are no conflicts of interest.

References

1. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med 2005;352:804-15.

2. Call TG, Norman AD, Hanson CA, Achenbach SJ, Kay NE, Zent CS, *et al*. Incidence of chronic lymphocytic leukemia and high-count monoclonal B-cell lymphocytosis using the 2008 guidelines. Cancer 2014;120:2000-5.

3. Zenz T, Döhner H, Stilgenbauer S. Differential diagnosis, staging, and prognostic factors. In: O'Brien S, Gribben JG, editor. Chronic Lymohocytic Leukemia. Vol. 7. USA: Informa Healthcare, Inc.; 2008. p. 103-20.

4. Chang CC, Liu CZ, Cleveland R. Prognostic implications of CD38 associated markers expression in B-CLL. Blood 2000;96:372A.

5. Malavasi F, Deaglio S, Damle R, Cutrona G, Ferrarini M, Chiorazzi N, *et al*. CD38 and chronic lymphocytic leukemia: A decade later. Blood 2011;118:3470-8.

6. Chiorazzi N. Implications of new prognostic markers in chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program 2012;2012:76-87.

7. Ottaggio L, Viaggi S, Zunino A, Zupo S, Rossi E, Spriano M, *et al*. Chromosome aberrations evaluated by comparative genomic hybridization in B-cell chronic lymphocytic leukemia: Correlation with CD38 expression. Haematologica 2003;88:769-77.

8. Kipps TJ. Chronic lymphocytic leukemia and CD38 related with IgD and IgM. In: Kaushansky K, Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Prchal J, editors. Williams Hematology. 8th ed. China: The McGraw-Hill Companies, Inc.; 2010. p. 94.

9. Matutes E, Morilla R, Morilla AM. Immunophenotyping. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors. Dacie and Lewis Practical Haematology. 11th ed. China: Churchil Livingstone/Elsevier; 2011. p. 353-66.

10. Jasim HN, AL-Mudallal SS. Immunohistochemical Expression of Bcl2 and Ki67 in Chronic Lymphocytic Leukemia (CLL). M.Sc. Thesis (Path) Al-Nahrain University; 2010.

11. Sorour A, ElSorady M. Expression of CD38 and ZAP-70 in chronic lymphocytic leukemia and their relation to clinical stage. J Med Res Inst 2007;28:174-80.

12. Mohammed S, Abid SA, AL-Rubaie HA. Immunohistochemical Analysis of Bcl-2 to Assess the Signifi cance of Dysregulated Apoptosis to Evaluate Angiogenesis in Chronic Lymphocytic Leukemia. M.Sc. Thesis (Path) University of Baghdad; 2010.

13. Karen W, Susan M, O'Brien MD. Chronic lymphocytic leukemia: Diagnosis and clinical features of CLL. Mayo Clin Proc 2006;81:1105-29.

14. Cantu ES, McGill JR, *et al*. Staging system detected in a population of chronic lymphocytic leukemia patients. Hematol Rep 2013;5:13-7.

15. Ja'afar AM, Abdulmajeed BA, AL-Rubaie HA. Flowcytometery Analysis of p-53 and Bcl-2 Oncogenes and Angiogenesis Factors VEGF and CD38 in Chronic Lymphocytic Leukemia. Ph.D. Thesis (Path) University of Baghdad; 2008.

16. Thunberg U, Johnson A, Roos G, Thörn I, Tobin G, Sällström J, *et al*. CD38 expression is a poor predictor for VH gene mutational status and prognosis in chronic lymphocytic leukemia. Blood 2001;97:1892-4.

17. Shen PU, Fuller SG, Rezuke WN, Sherburne BJ, DiGiuseppe JA. Laboratory, morphologic, and immunophenotypic correlates of surface immunoglobulin heavy chain isotype expression in B-cell chronic lymphocytic leukemia. Am J Clin Pathol 2001;116:905-12.

18. Smith A, Howell D, Patmore R, Jack A, Roman E. Incidence of haematological malignancy by sub-type: A report from the Haematological Malignancy Research Network. Br J Cancer 2011;105:1684-92.

19. Kar R, Kumar R, Tyagi S. De-novo CD5+B-prolymphocytic leukemia (PLL) presenting at younger age with favourable outcome. Turk J Haematol 2008;25:149-51.

20. Matrai Z, Lin K, Dennis M, Sherrington P, Zuzel M, Pettitt AR, *et al*. CD38 expression and Ig VH gene mutation in B-cell chronic lymphocytic leukemia. Blood 2001;97:1902.