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Assessment of beta-2 microglobulin and CD49d in patients with chronic lymphocytic leukemia pre- and posttherapy

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Abstract:

BACKGROUND: Chronic lymphocytic leukemia (CLL) is the most common type of leukemia among adults in Western countries; however, it is relatively rare in Asia. It is characterized by abnormal proliferation of lymphocytes in the blood, bone marrow, and lymphatic tissue. The measurement of serum beta-2 microglobulin (B2M) is essential for baseline workup of multiple myeloma and follicular lymphoma patients. CD49d, an adhesion molecule mediating cell-to-cell and cell-to-extracellular matrix interactions, represents a novel and the most reliable immunophenotypic marker regarding prognosis and independent of other markers.

OBJECTIVES: This study aimed to assess the level of B2M and CD49d in serum CLL patients and correlates them with treatment response.

PATIENTS AND METHODS: this is a prospective cohort study conducted on 70 patients with CLL and 40 healthy people as a control group. Patient groups were divided into two groups: The first group included 38 patients before receiving treatment and the second group included 32 patients posttreatment. Diagnosis was based on lymphocyte count of >5 × 109/L and immunophenotyping. The measurement of level B2M and CD49d in serum patients was done using enzyme-linked immunosorbent assay.

RESULTS: there were 53 males and 17 females, the mean age was 59.12 ± 14.23, and the most clinical presentation was lymphadenopathy. Regarding the mean of B2M, it was 2.19 ± 0.86, 1.86 ± 0.58, and 1.41 ± 0.44 in the pre, post, and control groups, respectively, with $P = 0.0001$. Regarding the mean of CD49d, it was 0.22 ± 0.15, 0.30 ± 0.44, and 0.19 ± 0.13 in the pre, post, and control groups, respectively, with $P = 0.211$.

CONCLUSION: this study showed that CD49d has no clinical impact on the treatment outcome, yet B2M has an important prognostic factor in deciding patients in advance stage.

Keywords:

Beta-2 microglobulin, CD49d, chronic lymphocytic leukemia

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia among adults in Western countries; however, it is relatively rare in Asia.[1] The median age at diagnosis ranges from 67 to 72 years, and the condition is more common in males.[1] The clinical course of CLL is highly variable, ranging from no symptoms to the rapid development of features of high-risk disease.[2] Lymph node swelling is the most common presenting feature of CLL, although fever, night sweats, and weight loss are sometimes seen. The most common physical findings are lymphadenopathy, splenomegaly, and less

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frequently, hepatomegaly.[2] A diagnosis of CLL can be established via a complete blood count (CBC) showing the progressive accumulation of clonal B-cells (>5000 B-lymphocytes/mL) over a period of at least 3 months and an immunophenotype study demonstrating clonal lymphocytes (LYM) expressing B-cell markers and cluster of differentiation (CD).[3] Both the modified Rai and Binet clinical staging systems are widely used to classify CLL patients into different prognostic groups according to the extent of lymph node involvement, enlargement of the liver and/or spleen, and blood findings (i.e., anemia and thrombocytopenia).[4] A number of clinical and biological features have been used to separate patients with CLL into subgroups with different prognoses and requirement of different therapeutic approaches. CD49d, CD38, and ZAP-70 expressions have been proposed as easily investigated markers (by flow cytometry) that have been shown to independently predict prognosis in CLL. CD49d, an adhesion molecule mediating cell-to-cell and cell-to extracellular matrix interactions, represents a novel and the most reliable immunophenotypic marker regarding prognosis and independent of other markers such as immunoglobulin heavy chain variable region gene(IgHV) mutational status.[5] Beta-2 microglobulin (B2M) is synthesized in all nucleated cells and forms the light chain subunit of the major histocompatibility complex class I antigen. Freely soluble B2M can be detected in blood, urine, and cerebrospinal fluid, following its release from the cell surface or cytoplasm.[6] Specifically, the measurement of serum B2M is essential for baseline workup of multiple myeloma and follicular lymphoma patients.[7] The measurement of Beta-2 Microglobulin (β2M) in serum is easy, fast, inexpensive, and highly reproducible. Serum β2M levels correlate with progression-free survival Patients with β2M levels <3.5 mg/L have a substantially longer time to progression than those with levels >3.5 mg/L.[8] Moreover, elevated β2M levels are associated with lower rates of complete remission and shorter failure-free and overall survival.[9] Adjusting the β2M level for creatinine clearance may improve its predictive ability.[10] This study aimed to assess the level of B2M and CD49d in patients' serum pretreatment and posttreatment and compared to the control group to monitor disease status and response to treatment.

Patients and Methods

This is a prospective cohort study carried out between December 2018 and December 2019 at Medical City Complex in Baghdad, Iraq. The diagnosis of patients included in this study was made by CBC and Blood film and flow cytometry on sample of peripheral blood or bone marrow. This study was approved by the Scientific and Ethical Committees of the College of Science, Al-Mustansiriyah University, Baghdad, Iraq. All patients who participated in this study were signed written informed consent prior to enrollment in the study.

Patients

This study was conducted on 110 samples: 40 controls and 70 patients divided into two groups – the first group was 38 pretreatment patients and the second group was 32 posttreatment between December 2018 and December 2019.

Laboratory parameters affect the disease

Hematological profile

The hematological parameters included in this study were: the total white blood cell counts (WBCs), Lym (lymphocytes), hemoglobin (HGB), and platelet count (PLT), these parameters were measured by The ADVIA® 560 and 560 AL Hematology systems (siemens Healthineers, Germany).

Determination beta-2 microglobulin and CD49d by enzyme-linked immunosorbent assay

Serum B2M and CD49d levels were determined using enzyme-linked immunosorbent assay (ELISA) MINDRAY Elisa Reader (MR-96A) radioimmunoassay kit (Immunotech, Prague, Czech Republic) according to the manufacturer's instructions.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 25.0 (IBM Corp., Armonk, New York, USA). A Chi-squared test was used to compare the associations between proportions. $P \leq 0.050$ was considered statistically significant.

Results

There were 70 patients diagnosed with CLL, the mean age was 59.12 ± 14.23, and there were 53 males forming 76%; regarding the stage of the disease, we found that B stage had 44 patients (63%), as shown in Table 1.

Regarding the hematology parameter, this study found that the mean level for WBC, HB, LYM percentage, and PLT were 40.98, 10.55, 59.12, and 186.44, respectively, in pretreatment, as shown in Table 2, with significant 0.0001 for all parameters.

We used the Chi-square test for independence to determine whether there is a significant association between the gender with treatment, gender with age groups, and treatment with age groups, as shown in Table 3.

Regarding the mean with SD for B2M, it was 2.19 ± 0.86, 1.86 ± 0.58, and 1.41 ± 0.44 in the pre, post, and control

groups, respectively. Moreover, in CD49d, it was 0.22 ± 0.15, 0.30 ± 0.44, and 0.19 ± 0.13 in pre, post, and control groups, respectively, as well as both the minimum and the maximum between all parameters for all indicators within the study, as shown in Table 4.

Discussion

Freely soluble B2M can be detected in blood, urine, and cerebrospinal fluid after its release from the cell surface or cytoplasm.[6] In particular, the measurement of B2M in blood is important for the primary action of patients with multiple myeloma and follicular lymphoma.[7] A number of clinical and biological characteristics have been used to subdivide CLL patients into subgroups with different expectations and requirements for different treatment approaches. Expressions of CD49d, CD38, and ZAP-70 have been suggested as easily investigated markers (by flow cytometry) that have been shown to independently predict prognosis in CLL. CD49d, an adhesion molecule that mediates interactions between cells and the extracellular matrix, represents a new and more reliable immune marker for prognosis and does not depend on other markers such as IGHV mutagenicity.[5]

The differences in hematological parameters in the current study results for patients pre-therapy group,and this indicates that the disease got different stages for patients depending on Binet classification. Some of patients were in stage B, which is characterized by the absence of severe anemia or a decrease in the number of platelets, while another group of patients presented with advanced stage (stage C), which are characterized by anemia and / or thrombocytopenia. These results and variations are consistent with Aldhahry et al study.[11] The total CLL patients enrolled in the present study were composed of 53 males (75.7%) and 17 females (24.3%) with higher incidence in males than in females (ratio 3:1), which was similar to other studies.[11,12]

Table 1: Demographic characteristics of patients and control group included in this study

Parameter	Pre	Post	Control
Age (years), mean±SD	60.45±14.16	57.26±14.38	61.12±14.23
Gender			
Male	28	25	18
Female	10	7	22
Binet stage			
A	0	0	
B	27	17	
C	11	15	

SD=Standard deviation

In contrast to Grygalewicz *et al*., the median age at the time of diagnosis was 62 years (range, 24–78). Fifty-five percent of the patients were male.[13] While Nedeva in his study that the percentage of males was 64%, while the percentage of females was 46%. As for the number of patients in stage B, it was 20%, while in phase C, it was 22%.[14] These differences in percentages from our study depend on the difference in the number of samples within the study as well as the difference in the geographical area and the country in which the study is conducted.

Rossi *et al*. reported CD49d expression as a risk factor of treatment-free survival in CLL patients.[15] In our study, a close association between CD49d and β2M is also described. It is generally believed that β2M is released constitutively by CLL cells and that its level approximately correlates with tumor mass.[12] B2M has been reported to be a growth factor and signaling molecule in several types of cancer cells (leukemia, lymphoma) and plays multiple roles in the development of cancer and promotes the formation of tumors and angiogenesis.[12,16] It is known that cancer growth is accompanied by an increase in serum concentration of many different soluble factors that are released into the bloodstream directly from tumor cells or indirectly from cells that are activated in response to the tumor. This study showed that the average serum β2M level before treatment was significantly higher in patients than in the control group. A high pretreatment of B2M is associated with lower survival results. B2M is a fixed peptide that is not covalently bound to Class I human leukocyte antigen molecule and is expressed on the surface of all nuclear cells. B2M is found free in body fluids under physiological conditions due to falls from the surface of cells or intracellular release[17] associated with the stage of the disease and tumor burden in patients with CLL.[16]

In addition, in patients who initially did not have indications for treatment, the increase in the B2M level at diagnosis was independently associated with a shorter survival after initiation of treatment. In current study, when comparing B2M concentrations

Table 2: Hematology parameters in pre, post, and control groups

Groups study	PLT	LYM	HB	WBC
Pre, mean±SD	186.44±80.37	59.12±19.58	10.55±2.65	40.98±40.97
Post, mean±SD	130.33±80.30	50.53±23.47	9.54±2.88	7.74±6.19
Control, mean±SD	297.22±113.85	32.57±8.79	12.83±2.21	6.62±2.17
Significant	0.0001	0.0001	0.0001	0.0001

PLT=Platelet count, LYM=Lymphocyte, HB=Hemoglobin, WBC=White blood cell, SD=Standard deviation

Table 3: Association of treatment with gender factor and association of age groups with gender and treatment subgroups

	Treatment			Total	*P*
	Pre	Post	Control		
Gender					
Male	27	24	18	69	0.014
Female	11	8	22	41	
Total	38	32	40	110	
	Age (years)				
	<50	50-59	60-69	≥70	
Gender					
Male	9	18	32	10	69	0.001
Female	17	14	7	3	41	
Total	26	32	39	13	110	
	Age				
	<50	50-59	60-69	<70	
Treatment					
Pre	7	8	17	6	38	0.204
Post	7	8	12	5	32	
Control	12	16	10	2	40	
Total	26	32	39	13	110	

Table 4: Beta-2 microglobulin and CD49d parameters in CLL patients and control groups

Groups study	CD49	B2M
Pre		
Mean	0.22	2.19
n	38	38
SD	0.15	0.86
Minimum	0.02	1.01
Maximum	0.58	3.96
Post		
Mean	0.30	1.86
n	32	32
SD	0.44	0.58
Minimum	0.05	0.58
Maximum	2.52	3.70
Control		
Mean	0.19	1.41
n	40	40
SD	0.13	0.44
Minimum	0.05	0.83
Maximum	0.62	2.57
Significant	0.211	0.0001

SD=Standard deviation, B2M=Beta-2 microglobulin

between the pre, post, and control groups, there was significant differences between these groups and these results are similar to that reported by Delgado et al., as it stated B2M remains a simple but very powerful predictor of treatment-free survival (TFS) and overall survival (OS) in patients with chronic lymphocytic leukaemia.[10]

The present study used measurement of CD49d by immunoassay to monitor the response to treatment as CD49d consider surrogate marker for progression of the disease in patients with CLL while previous studies measured the expression of CD49d on the surface of cells using flow cytometry. Our speculations by using immunoassay over flowcytomtry were to dcrease cost of the test and to make it readily available for all patients, so the results obtained from all groups and comparisons made between all study groups in table 4 as it shows that there were no significant differences between all study groups with the control group.

There are many studies refer to the importance of the immunomodulatory CD49d measurement by using flowcytomtry for CLL patients, e.g Haithem et al. reported that the expression of CD49d, CD38, and ZAP-70, which were detected in 60%, 56.7%, and 30% of patients, respectively. The correlations between the expression of CD38 and both CD49d and ZAP-70 were both statistically significant (P = 0.002). There was a statistically significant relationship between CD49d expression and Binet staging (P = 0.035), while no significant relations were found between both CD38 and ZAP-70 and Binet staging (P > 0.05). CD49d was more sensitive (76.5%) than the other two markers in predicting the intermediate and advanced stage with an accuracy of 70%.[5] Uzay *et al*. in Turkey reported that CD49d was expressed in 52% of CLL cases.[18] However, the result of this study was higher than that reported by by Gattei *et al*.[19] Rossi *et al*.[15] and Bulian *et al*.[20] as they showed that CD49d expression were 47%,39%,and 38% respectively. Moreover, this may be explained by the ethnic differences and the larger sample size of the other studies. CD49d expression showed a bimodal distribution, with most patients shown either very high or very low levels of expression. The mean percentage (range) of CD49d-positive expression was 44.74% (30%–75%), while for negative expression, it was 6.9% (0.5%–26%). This fact minimized the number of cases with borderline CD49d expression clustered around the cutoff, making CD49d a pragmatic choice of a biomarker for reliable prognostication of CLL.[20,21]

Conclusion

This study showed that CD49d has no clinical impact on the treatment using the ELISA method. We recommend using flow cytometry instead. B2M has an important prognostic factor in deciding patient in advance stage.

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Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Payandeh M, Sadeghi E, Sadeghi M. Survival and clinical aspects for patients with chronic lymphocytic leukemia in Kermanshah. Iran Asian Pac J Cancer Prev 2015;16:7987-90.
2. Abbott BL. Chronic lymphocytic leukemia: Recent advances in diagnosis and treatment. Oncologist 2006;11:21-30.
3. Hallek M. Chronic lymphocytic leukemia: 2017 update on diagnosis, risk stratification, and treatment. Am J Hematol 2017;92:946-65.
4. Hasan KM. Clinical aspects, immunophenotypic analysis and survival rate of chronic lymphocytic leukaemia patients in Erbil city. Iraq Sultan Qaboos Univ Med J 2018;18.E461-7.
5. Al-Rubaie HA, Thabit ZA, Jawad AM. Cd49d as prognostic marker in b-cell chronic lymphocytic leukemia in correlation with the expression of cd38, zap-70 and clinical Binet stage. Iraqi Postgrad Med J 2016;15:4.
6. Seo S, Hong JY, Yoon S, Yoo C, Park JH, Lee JB, *et al*. Prognostic significance of serum beta-2 microglobulin in patients with diffuse large B-cell lymphoma in the rituximab era. Oncotarget 2016;7:76934-43.
7. Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, *et al*. Revised international staging system for multiple myeloma: A report from international myeloma working group. J Clin Oncol 2015;33:2863-9.
8. Hallek M, Wanders L, Ostwald M, Busch R, Senekowitsch R, Stern S, *et al*. Serum beta (2)-microglobulin and serum thymidine kinase are independent predictors of progression-free survival in chronic lymphocytic leukemia and immunocytoma. Leuk Lymphoma 1996;22:439-47.
9. Wierda W. New prognostic factors in chronic lymphocytic leukemia. Clin Adv Hematol Oncol 2009;7:32-3, 42.
10. Delgado J, Pratt G, Phillips N, Briones J, Fegan C, Nomdedeu J, *et al*. Beta2-microglobulin is a better predictor of treatment-free survival in patients with chronic lymphocytic leukaemia if adjusted according to glomerular filtration rate. Br J Haematol 2009;145:801-5.
11. Al-Dahery HS, Alwan AF, Muslit HS. Estimation of zeta-chain-associated protein 70, tnterleukin-6 and interleukin-10 levels in sera of iraqi newly diagnosed chronic lymphocytic leukemia. Iraqi J Hematol 2016;5:173-7.
12. Ali IS, Al-Ani GA, Taher YM, Alwan AF. Prognostic values of beta 2 microglobulin, interleukin-4 and interleukin-6 in patients with different stage of chronic lymphocytic leukemia. Iraqi J Hematol 2020;9:47-50.
13. Grygalewicz B, Woroniecka R, Rygier J, Borkowska K, Rzepecka I, Łukasik M, *et al*. Monoallelic and biallelic deletions of 13q14 in a group of CLL/SLL patients investigated by CGH haematological cancer and SNP array (8×60K). Mol Cytogenet 2016;9:1.
14. Nedeva A, Naseva E, Kindekov I, Petkova N, Nikolov I, Raynov J. The prognostic significance of interphase cytogenetic abnormalities in chronic lymphocytic leukemia .Arch Hellen Med, 35,2018, 520-526.
15. Rossi D, Zucchetto A, Rossi FM, Capello D, Cerri M, Deambrogi C, *et al*. CD49d expression is an independent risk factor of progressive disease in early stage chronic lymphocytic leukemia. Haematologica 2008;93:1575-9.
16. Thompson PA, O'brien SM, Xiao L, Wang X, Burger JA, Jain N, *et al*. B2-microglobulin normalization within 6 months of ibrutinib-based treatment is associated with superior PFS in cell. Cancer 2017;122:565-73.
17. Xie J, Wang Y, Freeman ME 3rd, Barlogie B, Yi Q. Beta 2-microglobulin as a negative regulator of the immune system: High concentrations of the protein inhibit *in vitro* generation of functional dendritic cells. Blood 2003;101:4005-12.
18. Uzay A, Toptaş T, Kaygusuz I, Ekşioğlu-demiralp E, Tuğlular TF, Bayık M.The prognostic value of cd49d expression in Turkish patients with chronic lymphocytic leukemia. Turk J Hematol 2012;29:354-60.
19. Gattei V, Bulian P, Del Principe MI, Zucchetto A, Maurillo L, Buccisano F, *et al*. Relevance of CD49d protein expression as overall survival and progressive disease prognosticator in chronic lymphocytic leukemia. Blood 2008;111:865-73.
20. Bulian P, Shanafelt TD, Fegan C, Zucchetto A, Cro L, Nückel H, *et al*. CD49d is the strongest flow cytometry-based predictor of overall survival in chronic lymphocytic leukemia. J Clin Oncol 2014;32:897-904.
21. Popova VS, Blajeva SO, Alexandrova ML, Lukanov TH, Naneva SY, Tzvetkov NT. Prognostic biomarkers in early-stage b-CLL patients. J Biomed Clin Res 2017;10:2.