

Prognostic Significance of HLA-DR Expression in Sudanese Patients with Chronic Lymphocytic Leukemia

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Abstract

Background: The clinical course of patients with chronic lymphocytic leukemia (CLL) is heterogeneous, The Rai and Binet clinical staging systems are used to predict survival. An evaluation of prognostic factors at the time of diagnosis can guide the timing and strategy of treatment.

Aim: To evaluate the prognostic significance of HLA-DR in patient with CLL.

Method: Thirty five (27 male/ 8 female) newly diagnosed chronic lymphocytic leukemia (CLL) were analyzed using flow cytometry and finding were correlated with the clinical course of the disease.

Results: The mean of HLA-DR expression on subject were (38.1 ± 8.2) compared (79.1 ± 6.8) which was the mean of 35 normal volunteers tested at the same period of time. Analysis of the data revealed that the decrease of HLA-DR expression observed in stage 0 to IV was statistically significant (P.value < 0.00).

Conclusion: The present study demonstrated that the low HLA-DR expression correlate with stage III and IV Rai classification that indicate this parameter may play role in prognosis of disease.

Keywords: Chronic lymphocytic leukemia (CLL), HLA-DR, monocyte.

Introduction

Chronic lymphocytic leukemia (CLL) is a molecularly heterogeneous disease[1]. Is associated with immune suppression in the host which contributes to the failure to mount an effective immune response against the cancer cell [2]. typically affects elderly patients concomitant one or more pathological conditions or functional limitations with reduction of patient's life expectancy and represent major limitations in the adoption of standard therapies [3]. novel strategies for treatment and prognostication of chronic lymphocytic leukemia (CLL) evolve, the traditional Rai and Binet systems. Identification of novel prognostic markers of chronic lymphocytic leukemia such as immunoglobulin heavy-chain variable gene (IGHV) mutation status, leukemia-cell expression of CD38, ZAP-70, and CD49d, β-2-microglobulin, cytogenetic abnormalities detected by fluorescent in situ hybridization (FISH) and patient characteristics which include sex and age[4,5].

Rai stage 0: Lymphocytosis and no enlargement of the lymph nodes, spleen, or liver, and with near normal red blood cell and platelet counts[6].

Rai stage I: Lymphocytosis plus enlarged lymph nodes. The spleen and liver are not enlarged and the red blood cell and platelet counts are near normal

Rai stage II: Lymphocytosis plus an enlarged spleen (and possibly an enlarged liver), with or without enlarged lymph nodes. The red blood cell and platelet counts are near normal

Rai stage III: Lymphocytosis plus anemia (too few red blood cells), with or without enlarged lymph nodes, spleen, or liver. Platelet counts are near normal

Rai stage IV: Lymphocytosis plus thrombocytopenia (too few blood platelets), with or without anemia, enlarged lymph nodes, spleen, or liver [6].

Doctors separate the Rai stages into low-, intermediate, and high-risk groups when determining treatment options.

Stage 0 is considered low risk. Stages I and II are considered intermediate risk. Stages III and IV are considered high risk [6].

HLA-DR is an MHC class II cell surface receptor encoded by human leukocyte antigen complex on chromosome 6 .the complex HLA-DR (human leukocyte antigen –antigen

D related) and it is aligned. These cell surface proteins are responsible for regulation of immune system in human [7].

Human CD19 Antigen it is transmembrane glycoprotein located on the short arm of chromosome 16 which found on surface of B cell CD19 may play a role in controlling the progression of early pre-B to small, resting pre-B cells in the bone marrow. CD19 is one of the most reliable surface biomarker for B cells. It is expressed from pre-B cells until the terminal differentiation to plasma cells[8].

In previous studies reveal that: CD14+HLA-DRlow/-MDSCs was correlated with CLL tumor and a poor prognosis for CLL patients, and CD14+HLA-DRlow/-MDSCs were significantly correlated with the presence of CD4+ T and CD5+CD19+ cells , which could significantly inhibit the CD4+ T-cell immune response. They also study the relationship between the pretreatment CD14,HLA-DR cells and CD19 B cells (predominantly CLL cell) counts ; for these comparisons we converted the percentage of CD14 HLA-DR or monocyte into cell count there was positive correlation between B cell and both monocyte count and increase number of CD4 HLADR [9.]

Also in other study reveal the absolute monocyte count (AMC) reflects the monocyte-derived cells in the microenvironment, and elevates in AMC is associated with increased CLL cell survival in vivo AMC at diagnosis of CLL is correlated with clinical outcomes as time to first therapy and overall survival also in diagnosis and prognosis [10] and also An increase CD19+ lymphocyte count at diagnosis is marker of increasing risk of poor prognosis[11].

In present study measure concentration of HLA -DR expression and if correlation between prognosis of CLL or not. We used CD19 for exclude B lymphocyte and detected concentration of HLA-DR on monocyte.

Material and methods

Patient Population

After obtain informed consent, EDTA peripheral blood sample was collected from 35 patients 27 male 8 female (age range 42-90 years) previous diagnosed with CLL and 35 control samples collected from healthy individuals at flow cytometry center in Khartoum .

Case control study performed in flow cytometry center in Khartoum state in the period from 20 February 2016 to 30 may 2016 , and data obtained from patients by using standard questionnaire with ethical approval with ministry of health .

The diagnosis of CLL was confirmed in each patient by flowcytometry (EPICS XL Beckman Coulter flow cytometer, Miami ,FL,USA) using fluorescent dye labeled monoclonal antibody for CD5, CD10 ,CD19, CD20 ,CD22, CD23 , FMC7 and diagnostic score was determined , ZAP-70 and CD38 used as prognostic markers.

Clinical characteristics of patients including age, sex, and complete hemogram were performed including

hemoglobin, red blood cell count, platelet count, total white blood cell count with differential lymphocyte percentage.

Methods

Absolute count of HLA-DR were performed by flowcytometry according to the standard protocol from Beckman Coulter using flow cytometer EPICS XL by using monoclonal antibody labeled with fluorescent dyes : anti-HLA-DR fluorescein isothiocyanate (FITC), anti-CD19-phycoerythrin (PE) and using anti CD45- phycoerythrin – tandem dye (PEcy5) . CD45 is used for gating of leukocyte in patient population show figure (3,A) and control populations show in figure (3,B). and with CD19 to exclude B lymphocyte in control population show in figure (4,B) and patient population show in figure(5,B) which express HLA-DR in monocyte in control population show in figure(4,A) and patient population show in figure(5,A), The number of the HLA-DR cells was counted.

Statistical analysis

All data of study population and each parameter were analyzed by using parametric (frequencies, mean+/-SD, independent t-test and one way ANOVA and post hoc analysis were used to assess differences between and within the stages and treatment option risk by using Statistical Package for Social Science SPSS (v.20) statistical computer software program. Comparative analysis of means was performed with significant levels were set at (P. value < 0.05), and the results presented in form of Bars and tables.

Results

All 35 CLL patients in the study were classified by Rai stages (Table 2). The largest numbers of patients were in Rai stage IV (n = 10) and Rai stage 0 (n = 7).

The mean of HLA-DR of 35 patients (27 male/ 8 female) with CLL was 79.1 ± 6.8 which was the mean of 35 normal volunteers tested at the same period of time (p < 0.00). The expression of HLA-DR cells, according to the different stages of the disease are shown in figure (1, 2). Analysis of the data revealed that the decrease of HLA-DR ratio observed in stage 0to IV was statistically significant (P. value < 0.00).(Table 3). But when compare with normal control insignificant, when compared with stage (P. value > 0.48) (Figure 2)

Table 1: Patients characteristics

Haematological parameter	Mean	Std. Deviation
Age average	64.20	11.704
TWBCs (×10 ⁹ /L)	106.31	100.056
Lymphocyte(×10 ⁹ /L)	81.789	9.1884
RBCs (×10 ⁶ /L)	3.77	1.190
Haemoglobin (g/dL)	11.429	2.7684
Platelet(×10 ³ /L)	156.43	88.718

Table 2: Rai Classification

Rai Class	Frequency	Percent %
Stage 0	7	20.0
Stage I	6	17.2
Stage II	6	17.2
Stage III	6	17.2
Stage IV	10	28.4
Total	35	100.0

Table 3: HLADR expression among study group

	Sample	Mean	Std. Deviation	Std. Error Mean
HLADR	Case	38.1857	8.26595	0.000
	Control	79.1486	6.80041	0.000

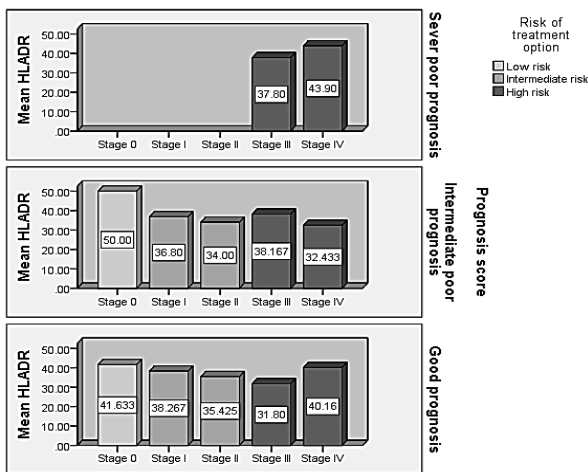


Figure1: Expression of HLA-DR among patient with different stages of Ria staging system of CLL P. value < 0.00 is significant between groups

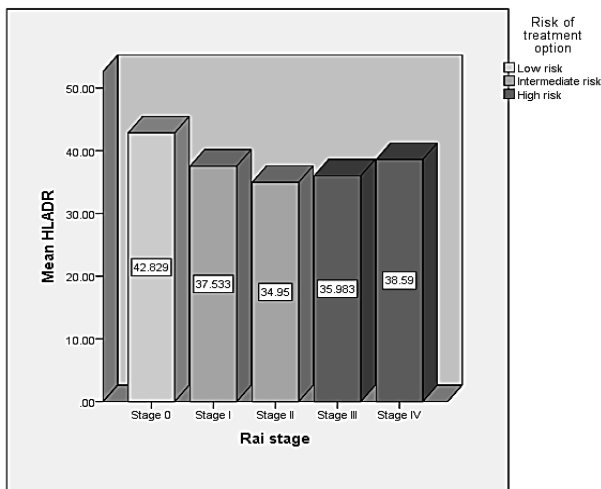


Figure2: Expression of HLA-DR among patient with different stages of CLL P. value < 0.48 is insignificant between group

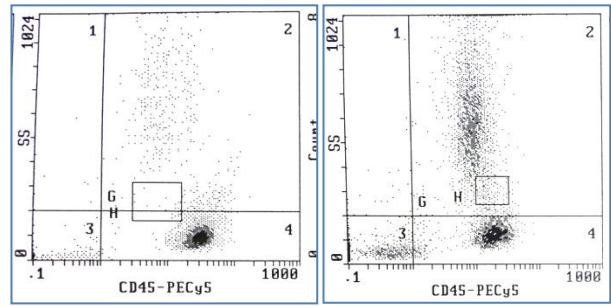


Figure 3: Gating leukocyte by SS and CD54

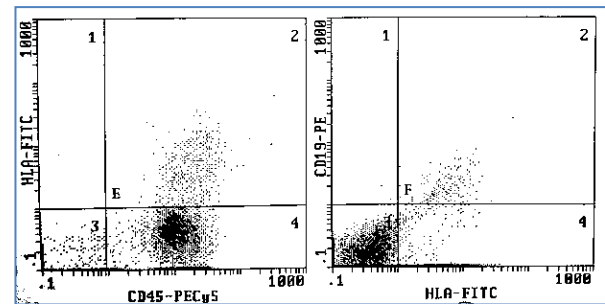


Figure 4: Expression of HLA-DR with CD45 and CD19 in control population

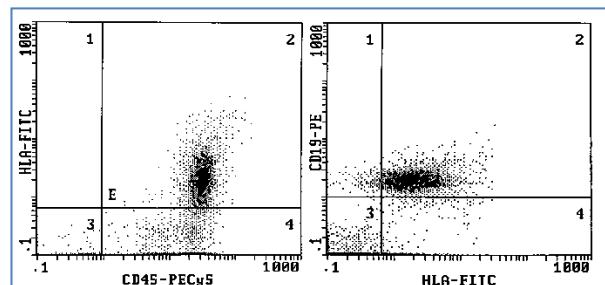


Figure 5: Expression of HLA-DR with CD45 and CD19 in patients population

Discussion

In our study show that the expression of HLA-DR in patients with CLL was decreased significantly with late stages of disease. We correlate the HLA-DR expression with Rai classification and show that the expression was decreased significantly in stage III and IV. In comparison with previous studies the results of current study agree with the previous report.

In conclusion in our knowledge the Population of immunosuppression monocyte characterized by CD14⁺/HLA-DR^{low} phenotype which was significantly elevated in CLL patient and were Predict survival in CLL patients.

Conclusion

The present study demonstrated that the HLA-DR expression correlate with stage III and IV Rai classification and that indicate this parameter may play role in prognosis of disease.

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