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Study Of Bax/Bcl-2 Ratio In Chronic Lymphocytic Leukemia Patients

Thesis

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SUMMARY

Chronic lymphocytic leukemia (CLL) is a clinically heterogenous disease characterized by the accumulation of mature appearing lymphocytes in the blood, marrow, lymph nodes and spleen. There is considerable heterogeneity in CLL in terms of cellular biology, molecular genetics and prognosis.

The mechanisms involved in the development of the disease suggest that the modulation of survival signals interfering with apoptosis may be an important tool in the pathogenesis of CLL. Even though dysregulation of apoptosis is a distinctive feature of CLL, none of the apoptotic defects has been found to be universally present among CLL patients. This may explain the heterogeneity of the clinical course and response to therapy in CLL.

A number of proteins play a role in apoptosis which may be altered in CLL. Bcl-2 family of antiapoptotic (Bcl-2) and proapoptotic (Bax) proteins are critical regulators of apoptosis in CLL and their deregulated function is involved in the pathogenesis and progression of CLL.

In CLL, malignant clonal B cells accumulate because of dysregulated production and inappropriately prolonged survival due to impairment of apoptosis. In fact, the alteration of some apoptotic mechanisms such as the balance between the proapoptotic (Bax) and anti-apoptotic (Bcl-2) members of the Bcl-2 family is recognized to be an important factor not only in the pathogenesis but also in the development of chemorefractoriness in CLL.

The present study was carried on 50 newly diagnosed patients with chronic lymphocytic leukemia presented to the Hematology/ Oncology unit, Internal Medicine department, Tanta University Hospital. The studied patients were 30 males and 20 females with a male to female ratio 1.5:1 and their ages ranged from 39 to 76 years.

The aim of the study was to estimate the Bax/Bcl-2 ratio in chronic lymphocytic leukemia patients and its association with clinical features and disease characteristics and some prognostic markers for therapeutic significance and clinical management of the disease.

The patients were diagnosed on the basis of the presence of > 5 $\times 10^9$/L monoclonal B-lymphocytes by immunophenotyping on peripheral blood samples. The patients were classified according to the modified Rai staging system into 3 subgroups where the low risk patients included 11 patients (22%), intermediate risk patients included 26 patients (52%) and the high risk patients were 13 patients (26%).

Lymphadenopathy was the commonest presentation among the studied patients and was found in 44% of patients. Splenomegaly was present in 42% of patients and only 14% of patients presented with hepatomegaly.

All patients were subjected to full history taking, thorough clinical examination with emphasis on hepatomegaly, splenomegaly and lymphadenopathy and routine laboratory investigations which included complete blood count with examination of peripheral blood film, LDH, liver and renal function tests. Immunophenotyping on peripheral blood samples for diagnosis of CLL using the panel used for chronic leukemia was done for all cases.

Immunophenotyping on peripheral blood sample was done for detection of Bax and Bcl-2 using monoclonal antibodies by flow cytometry. Bax and Bcl-2 mean fluorescence intensity (MFI) were measured, and the ratio between them was calculated.

The median Bax/Bcl-2 ratio was 1.20. The difference between groups was statistically significant. The median Bax/Bcl-2 ratio was chosen as cut off to discriminate subgroups of cases, being very similar to the best cut off defined by ROC analysis. A ratio higher than 1.20 was associated with low Rai stage and good prognosis while ratio lower than or equal to 1.20 was associated with intermediate and high Rai stage and worse prognosis.

As regards the association of Bax/Bcl-2 ratio with hematological parameters, there was a significant positive correlation between Bax/Bcl-2 ratio and hemoglobin level and platelet count. While, there was a significant negative correlation between Bax/Bcl2 ratio and age also with total WBCs count and absolute lymphocytic count.

No significant correlation was found between Bax/Bcl-2 ratio and urea nor creatinine. The liver enzymes (ALT and AST) did not show any correlation with Bax/Bcl-2 ratio.

Serum LDH was significantly higher with stage progression and there was a significant negative correlation between Bax/Bcl-2 ratio and LDH.

A significant relationship was found between Bax/Bcl-2 ratio and some well-known biological prognosticators. The expression of CD49d

and CD38 was significantly increased in CLL patients with disease progression. CD49d % and CD38 % were negative (less than 30%) in low risk patients and positive (equal and more than 30%) in all intermediate and high risk patients. The difference between groups was statistically significant. There was a significant negative correlation between Bax/Bcl-2 ratio and CD38 and CD49d. Also, lower Bax/Bcl-2 ratio was significantly associated with higher CD38 and CD49d .

Analysis of survival using Kaplan-Meier curve showed a significant higher overall survival in patients with Bax/Bcl-2 ratio positive (>1.2) than Bax/Bcl-2 ratio negative (≤1.2) patients and observed significant shorter overall survival in patients with lower Bax/Bcl-2. This study highlights the importance of investigating the expression of Bax/Bcl-2 ratio as an evaluating marker for patient prognosis.