Immunohistochemical study of p-53 protein expression in chronic lymphocytic leukemia and its correlation with clinicopathological factors

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Abstract:

**Background:** several factors render chronic lymphocytic leukemia an interesting subject for study by researchers. These include marked progress in understanding the molecular biology of normal and neoplastic lymphoid cells and recent advances in molecular genetics techniques. Among molecular markers, p-53 cancer suppressor gene has been widely studied.

**Objectives:** is to correlate p-53 protein expression in chronic lymphocytic leukemia, as examined by immunohistochemical method, with some pathological and clinical parameters.

**Patients and methods:** this is a retrospective study; whereby archival paraffin-embedded bone marrow tissue blocks along with the clinical and hematological records of fifty patients (35 males and 15 females), with chronic lymphocytic leukemia and twenty cases as control were obtained from the Department of Hematology of the Medical City Teaching Laboratories in the period from April 2012 to April 2014. P-53 was studied by immunohistochemical staining.

**Results:** the frequency of p53 positive patients in the study group was 16% (8 of 60 cases). Patients with high score for p-53 were more frequently and significantly associated with high-risk clinical stage than patients with low score. There was a significant direct positive correlation between increasing scores of p53-positive chronic lymphocytic leukemia cells and advancing clinical stage of the disease.

**Conclusion:** although p53 alteration may occur early in the course of the disease, as shown by the p53 positivity in a proportion of patients in low and intermediate-risk stage of the disease, the highest frequency p53-positive cells, has been observed in high-risk stage of the disease.

**Key words:** Chronic lymphocytic leukemia; P-53; Immunohistochemistry.

Introduction:

P-53 gene, located on chromosome 17 band p13.1, is frequently mutated in a wide variety of human tumors, including multiple myeloma. It encodes a 53-kD phosphoprotein that is normally present in the nuclei of the cells. (1-3) The p-53 tumor suppressor protein is a transcription factor that is involved in the cell cycle arrest, and induction of apoptosis in genetically damaged cells. Structural alterations and point mutations of the p-53 tumor suppressor gene occur in variable frequencies in chronic lymphocytic leukemia (CLL); they have been associated with poor survival and nonresponse to therapy, suggesting that p-53 may play a role in the clinical course of the disease.4

**Patients and methods:**
This is a retrospective study, whereby archival paraffin-embedded bone marrow (BM) blocks along with the clinical and hematological records of fifty patients with CLL were obtained from the Department of Hematology of the Medical City Teaching Laboratories in the period from April 2012 to April 2014. The study include 35 males and 15 females, ages ranged between 48 and 82 years. The patients were newly diagnosed and did not receive prior treatment. The BM biopsies were performed at diagnosis. All relevant clinical and laboratory data available for all patients were reviewed, and the peripheral blood and marrow aspirate smears (stained with Leishman stain) were re-examined carefully. BM biopsy sections stained with H & E were also re-examined. The following data were documented for each of the patients included: age, sex, the presence of lymphadenopathy, hepatomegaly, splenomegaly, PCV, total leucocyte count, platelet count, absolute lymphocyte count, % of prolymphocytes, absolute neutrophil count, bone marrow aspirate findings, especially percentage of lymphocytes, and pattern of bone marrow infiltration on bone marrow biopsy. The BM histology pattern (non-diffuse or diffuse) was evaluated according to Rozman et al. [Rozman et al. 1984]:5 Four different patterns were recognized according to the following criteria:

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1. Interstitial: Some degree of replacement of normal hemopoietic tissue by mature lymphocytes is observed, but with preservation of fat cells and bone marrow structure.
2. Nodular: Nodules made up of mature lymphocytes appear. There is no interstitial infiltration. Fat cells are preserved.
3. Mixed: A combination of interstitial and nodular patterns.
4. Diffuse: Diffuse lymphoid infiltration with massive replacement of normal hemopoietic tissue as well as fat cells.

For the present analysis, patterns 1, 2, and 3 were pooled as a nondiffuse group.CLL patients were diagnosed and selected according to the criteria of the International Workshop on CLL (IWCLL), which included: 1- Persistent absolute lymphocytosis of more than 10,000 mature-appearing lymphocytes/µL in the peripheral blood, 2- bone marrow aspirate smear with lymphocytes ≥ 30% of all nucleated cells and 3- B-cell phenotype of peripheral blood. Criteria 1 with either 2 or 3 were needed for diagnosis. In this study diagnosis was based on criteria 1 and 2. All patients had peripheral blood prolymphocytes of less than 10%.

Clinical staging was done according to the modified Rai staging system:7
Low-risk patients: With only lymphocytosis in the PB and BM (stage 0).
Intermediate-risk patients: With lymphocytosis and lymphadenopathy (stage I) and/or hepatosplenomegaly (stage II).
High-risk patients: With lymphocytosis together with anemia (Hb < 11 g/dL) (stage III) and/or thrombocytopenia (Platelets < 100 × 109/liter).

Paraffin-embedded BM blocks of twenty age and sex matched control individuals (14 males and 6 females), ages ranged between 52 and 78 years, with their hematological reports were also collected. All the control BMs were negative for infiltrative lesions and were obtained from patients with anemia. All paraffin-embedded BM blocks (patients and control) were subjected to immunohistochemical staining for p-53 antigens in the histopathology department of the Medical City Teaching Laboratories.

Immunohistochemistry: serial 5-µm sections of the diagnostic BM biopsy were cut, deparaffinised and heat-induced antigen retrieved. The CLL cells were immunostained for p-53, DO-7 (DAKO, Glostrup, Denmark), at a 1:50 dilution using immunoperoxide reagents and Diaminobenzidinetetrahydrochloride (DAB) (BioGenex, laboratories, San Ramon, CA). A paraffin biopsy of squamous cell carcinoma of the skin specimen served as a positive immunohistochemistry control. For the negative control, all reagents were added except the diluted primary antibody. The percentage of p53 immunostained nuclei was evaluated microscopically under ×400 and ×1000 magnification (the percentage of p-53 positive lymphocytes was determined by counting 500 mature looking lymphocytes in different visual fields). The extent of P-53 positivity was interpreted as high if more than 10% of the tumor cells/HPF showed nuclear brownish staining, while low extent mean positive detection in less than 10% cells using HPF.8

Statistical analysis was performed with the SPSS 20 statistical software program. Univariate data were summarized using standard descriptive statistics. Associations between categorical variables were assessed via cross tabulation and chi-square. ANOVA and t-test were used to compare means of continuous variables. Spearman correlation was used to measure the association between two continuous variables or when at least one variable was ordered. Exact tests were used to calculate the p value. In all statistical analyses, a p value < 0.05 was considered to indicate statistically significant difference.

Results:
Fifty patients with CLL, 35 males and 15 females were included in this study. Ages ranged between 48 and 82 years. The mean age was 66.06 (± 9.4) years with a M:F ratio of 2.3:1.

Based on the immunohistochemical (IHC) staining pattern, the 50 patients were divided into two groups: 1. P-53-positive [n = 8 (16 %)] (Figure-1), and 2. P-53-negative [n = 42 (84 %)].

Figure 1. P-53 positive IHC nuclear staining on a BM biopsy of CLL (arrow), (×1000).

In the p-53 positive cases, three patients have a score less than 10% (low grade) while five patients have a score more than 10% (high grade). There were no significant differences in age and hematological parameters between p-53 positive and p-53 negative cases as shown in table 1.
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There was no significant association between p-53 expression and clinical stages of disease (Table 2). However, a significantly larger proportion of patients with high p-53 grade (p-53 positive cases) were associated with advanced clinical stage (intermediate and high stages) of the disease than patients with low p-53 grade (p = 0.018, table-3). At the same time, there was a significant positive linear correlation between increasing p-53 score (p-53 positive cases) and advancing clinical stage of the disease (p = 0.007, figure-2).

### Table 1. Patient’s characteristics according to p-53 expression in CLL patients

| Immunohistochemical result for P-53 | p         |
|------------------------------------|-----------|
| Patient’s characteristics          | Positive(N = 8) | Negative(N = 42) |
| Age                               | 60.13 ± 9.48 | 67.19 ± 9.06      | N.S           |
| Lymphocyte % (BM)                 | 72.63 ± 19.96 | 82.62 ± 13.4      | N.S           |
| PCV                               | 33.72 ± 5.07  | 31.57 ± 6.71      | N.S           |
| Platelet count(×10^9/L)           | 102.9 ± 48.4  | 94.64 ± 47.37     | N.S           |
| Absolute lymphocyte count (×10^9/L) | 73.13 ± 14.8 | 79.6 ± 13.8       | N.S           |

There was no significant association between p53 expression and BM infiltration pattern (Table 2) and between p-53 score and BM infiltration pattern (Table 3).

### Table 2. Associations between p-53 expression and pathological characteristics of CLL patients

| Immunohistochemical result for P-53 | p         |
|------------------------------------|-----------|
| Patient’s characteristics          | Positive(N = 8) | Negative(N = 42) |
| Modified Rai stage                 | Low 1/8 (12.5 %) | 2/42 (4.8 %)      | N.S           |
| BM infiltration pattern            | Diffuse 7/8 (87.5 %) | 24/42 (57.1 %)    | N.S           |
|                                   | Non-diffuse 1/8 (12.5 %) | 18/42 (42.9 %)    |               |

| Modified Rai stage | Low 1/3 (33.3 %) | 0/5 (0 %) |
|                   | Intermediate 2/3 (66.7 %) | 0/5 (0 %) |
|                   | High 0/3 (0 %) | 5/5 (100 %) |

Discussion:

Structural alterations and point mutations of the p-53 tumor suppressor gene have been shown in 10 % to 15 % of CLL; they have been associated with poor survival and nonresponse to therapy, suggesting that p-53 may play a role in the clinical course of the disease.(1,9,10) Immunohistochemistry has provided an objective method for assessing P-53 that was shown to be a significant prognostic indicator of various malignancies including CLL.(1- 4) The frequency of p-53 protein expression detected by immunohistochemistry in CLL patients in this study was 16 %, which was in accordance with other national (11,12) and international studies (1,9,10,13) that varies from 10-15 %. The coexistence of p-53-positive and -negative cells within the same leukemic population supports the hypothesis that p-53 dysregulation can be a late event in the progression of the disease. Although the p-53 alteration may occur early in the course of the disease, as shown by the p-53 positivity in a proportion of patients in low and intermediate-risk stage of the disease, the highest frequency p-53-positive cells, has been observed in high-risk stage of the disease (table-2). These findings are in agreement with previous studies, which have shown a strong correlation between p-53 mutations and progression in hematologic malignancies including CLL. (1,9,10) Similar results were reported in Iraq.(11,12) No significant differences were found in hematological parameters between p-53-positive and p-53-negative patients (Table 1). Other national (11,12) and international studies (1,9,10,13) reported similar results. There was no significant association between p-53 expression and clinical stages of disease (Table 2). However, this study revealed that p-53 score is significantly correlated with the clinical stage of the disease (Table 3 and figure 2), and thus it is an important prognostic variable in patients with CLL. These data concur with those reported by
other workers, in their analyses of p-53 score in patients with CLL. These investigators reported that significantly larger proportion of their p-53-positive patients are associated with advanced clinical stage of the disease than patients in early clinical stages. They also showed that the score of p-53 protein expression, as measured by immunohistochemistry was strongly correlated with p-53 gene mutations, advanced disease, progressive disease, refractoriness to therapy and reduced survival. (1, 9, 13) Similar results were reported in Iraq.(11,12) There was no significant association between p-53 expression, score, and BM infiltration pattern. Similar results were reported by national (10, 11) and international studies. (1,10,14) Regarding the forty-two p-53-negative CLL cases, the IHC procedure cannot exclude p-53 gene deletion in CLL cells. This deletion can be confirmed by the application of FISH technique on chromosomal preparations. The absence of a positive fluorescent signals confirm gene deletion.(15,16)

Author contribution:
Abdulrazzaq Wahhab Abdullah: Postgraduate (M.Sc.) Student: Study conception, Acquisition of data and Interpretation of data.
Abdulkareem Mohammad Jaafar: Supervisor: Study design, Data analysis, Drafting of manuscript and Critical revision.
Adel Rabeea ALsaadawi: Consultant Supervisor: Interpretation of data and Drafting of manuscript

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