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Prognostic Significance of P53, Bcl-2, and Fas Expression in Patients with Primary Gastrointestinal Diffuse Large B-Cell Lymphoma

ORIGINAL INVESTIGATION

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ABSTRACT

Objective: P53, Bcl-2, and Fas proteins play significant roles in lymphoid cell apoptosis. These proteins affect the prognosis and treatment response of lymphoma and various malignancies. The aim of the present study was to investigate the effects of P53, Bcl-2, and Fas protein expression on treatment and prognosis in patients with primary gastrointestinal diffuse large B-cell lymphoma.

Materials and Methods: Thirty-nine patients with primary gastrointestinal diffuse large B-cell lymphoma were included in the study. Immunohistochemical staining was performed to analyze P53, Bcl-2, and Fas protein expression levels in paraffin sections.

Results: We examined 39 patients with primary gastrointestinal diffuse large B-cell lymphoma, 21 males and 18 females, with a median age of 54 years. P53 protein expression was detected in 24 patients (61.5%), Bcl-2 protein expression was detected in 26 (67%), and Fas protein expression was detected in 28 (72%). The five-year overall survival rate was significantly lower in patients with P53 and Bcl-2 expression; on the other hand, we did not find a significant difference in the five-year overall survival with respect to Fas protein expression.

Conclusion: We found that P53 and Bcl-2 protein expression had a negative effect on prognosis and survival in patients with primary gastrointestinal diffuse large B-cell lymphoma. However, Fas protein expression had no effect on prognosis and survival. Taken together, patients with P53 and Bcl-2 expression should be considered to have a high risk from the beginning, and these patients should undergo aggressive treatments.

Keywords: Lymphoma, P53, Bcl-2, Fas, prognosis

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INTRODUCTION

Non-Hodgkin lymphomas (NHLs) vary in terms of clinical findings, histology, immunophenotypic features, treatment response, and prognosis. Depending on the increasing number of etiological causes in the past years, the incidence of extranodal lymphomas, especially aggressive gastrointestinal lymphomas, has increased (1). NHLs constitute 4% of all cancers and are responsible for 4%–6% of cancer-related deaths. In the United States, extranodal lymphomas constitute 15%–25% of all lymphomas in adults, whereas this rate increases to 40%–50% in Europe and Far East countries. In Turkey, the incidence of primary extranodal lymphomas ranges between 25% and 46%. Gastrointestinal lymphomas constitute 30%–40% of primary extranodal lymphomas. Diffuse large B-cell lymphoma (DLBCL) represents the most common histological subtype. The most common gastrointestinal involvements are the stomach, small intestine, colon, rectum, and esophagus (2-4). The current study investigated prognostic factors and the effect of apoptotic proteins P53, Bcl-2, and Fas in patients with DLBCL.

MATERIALS and METHODS

The medical records of patients who were followed-up with primary gastrointestinal DLBCL diagnosis at Erciyes University Medical Faculty Hematology clinic between January 1992 and September 2003 were retrospectively evaluated. Preparates and paraffin blocks of the patients were obtained from the archives of the Erciyes University Faculty of Medicine, Department of Pathology. Thirty-nine primary gastrointestinal patients with DLBCL with sufficient clinical and laboratory findings were included in the study. Twenty-one patients were males and 18 were females. The patients' ages ranged between 23 and 83 years. Prognostic factors including age, gender, performance status (ECOG), B-symptom presence, lactic acid dehydrogenase (LDH) level, Ann Arbor stage, extranodal involvement number, International Prognostic Index (IPI) score, bone marrow involvement, treatment and treatment response, and overall survival were recorded. In Ann Arbor staging, stage I and stage II were considered as early stages, whereas stage III and stage IV were considered as late stages. IPI variables used in IPI are age (<60 vs. >60 years), performance status (ECOG 0 or 1 vs. 2 to 4), Ann Arbor stage (I or II vs. III or IV), serum LDH level

(normal vs. increased), and extranodal involvement for patients older than 60 years. Disease staging and treatment response were evaluated using standardized data including bone marrow biopsy and abdominal and thorax tomography. According to the medical records of the patients, 29 underwent chemotherapy and surgery, eight underwent chemotherapy+radiotherapy+surgery, and two underwent only chemotherapy. First-line chemotherapy treatment was with cyclophosphamide, doxorubicin, vincristine, and prednisolone. Second-line treatments included methotrexate, folinic acid, doxorubicin, cyclophosphamide, vincristine, dexamethasone, and bleomycin; etoposide, cytarabine, cisplatin, and methylprednisolone; and dexamethasone, cytarabine, and cisplatin.

Complete remission was defined as the recovery of physical examination and radiological findings four weeks after the last round of chemotherapy. Partial remission was defined as a 50% reduction in initial tumor mass. Patients who did not comply with these criteria were considered as non-responders.

Immunohistochemical staining

Formalin-fixed paraffin-embedded tissue blocks were stained with Fas, Bcl-2, and P53 in 5-micron sections. The tissue sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. Heat-induced epitope retrieval was performed, and antigen retrieval was obtained in a microwave oven. We used a monoclonal antibody specific for Fas, Bcl-2, and P53 (all from Neomarkers, Fremont, CA, USA). All immunohistochemical protocols were manually performed, and a biotin–streptavidin detection system was used. The breast, tonsilla, and stomach were used as positive controls for the immunohistochemical staining of Fas (APO 1/CD 95), Bcl-2, and P53, respectively. Expression was semi-quantitatively scored by two observers (B.E and O.C). Five hundred tumor cells were counted for each patient. Positivity was defined as being equal to or greater than 10% positive cells. However, almost all negative tumors were completely negative or had equal to or fewer than 9% positive tumor cells.

Statistical analysis

The clinical parameters and outcomes were retrospectively reviewed. Prognostic data were analyzed according to known clinical parameters and Fas, Bcl-2, and P53 protein expression. Survival was estimated using the Kaplan–Meier method. A log-rank test was applied to analyze significant prognostic factors. Factors that proved to be statistically significant in the univariate analysis were also submitted to a multivariate analysis using the Cox proportional hazards model, which estimates the odds ratio in 95% confidence intervals to determine independent prognostic factors (enter and remove limits 0.1). Factors included Ann Arbor staging (I or II vs. III or IV), ECOG performance status 0 or 1 vs. 2–4, IPI score low (low vs. low-intermediate) and high (high-intermediate and high), bone marrow involvement (negative vs. positive), age (<60 vs. >60 years), B symptoms (negative vs. positive), extranodal involvement (0-1 vs. ≥2), LDH level (increased vs. normal), and P53, Bcl-2, and Fas (negative vs. positive). The relationship between complete remission and clinical and/or immunohistochemical parameters was evaluated using the χ^2 test. All P-values were based on two-tailed statistical analysis. P-values below 0.05 were considered to be significant. All analyses were performed using SPSS 10.0 statistical software (SPSS 10.0, Inc., Chicago, IL, USA).

Table 1. General characteristics of patients

Clinical characteristics	No	%
Gender		
Male	21	54
Female	18	46
Age (years)		
≤60	24	61.5
>60	15	38.5
Performance status (according to ECOG criteria)		
0-1	25	59
2-4	16	41
Ann Arbor stage		
I or II	21	54
III or IV	18	46
B symptoms		
Present	25	64
Absent	14	36
Extranodal involvement		
≤1	34	87
≥2	5	13
IPI score		
Low or low-intermediate		67
High-intermediate or high	13	33
Bone marrow involvement		
Present	5	13
None	34	87
LDH level		
Increased	18	46
Normal	21	54

ECOG: Eastern Cooperative Oncology Group; IPI: International Prognostic Index; LDH: lactate dehydrogenase

RESULTS

Twenty-one patients (54%) were males and 18 (46%) were females. The median age was 54 years (range: 23–83 years). The mean follow-up period was 17 months (range: 2–143 months). The general features of the patients are shown in Table 1.

Stomach involvement was seen in 71% of the patients, small intestine involvement in 21%, multiple involvement in 5%, and colon involvement in 3%. Complete remission was observed in 19 patients (49%).

The complete remission rate was significantly higher in male patients with good performance score, low Ann Arbor stage (stage I or II), normal LDH level, lack of B symptoms, 0 or 1 extranodal involvement, low IPI score (low or low-moderate), absence of bone

involvement, and lack of P53 expression ($p<0.05$). We did not find a significant correlation between complete remission and Bcl-2 and Fas protein expression levels.

P53 and Bcl-2 protein expression levels were significantly higher in patients with low IPI score (low and low-moderate) and high Ann Arbor stage (stage III or IV); in contrast, we did not find any significant correlation between Fas protein expression and these prognostic features. The five-year overall survival rates with respect to P53, Bcl-2, and Fas expression were 17%, 23%, and 33%, respectively. On the other hand, the five-year overall survival rates were 68%, 59%, and 36% in patients with no P53, Bcl-2, and Fas expression. The five-year overall survival rate was significantly lower in patients with P53 and Bcl-2 expression; on the other hand, we did not find a significant difference in the five-year overall survival rate with respect to Fas protein expression.

The univariate analysis showed that the overall survival rate was significantly lower in patients with poor performance status, high Ann Arbor stage (stage III or IV), two or more extranodal involvements, high IPI score (high-moderate and high), and P53 and Bcl-2 protein expression. The multivariate analysis showed that P53 expression was an independent prognostic factor for overall survival.

DISCUSSION

Apoptosis, also known as programmed cell death, is a type of physiological cell death. Mutations in apoptosis regulator genes prevent cells from undergoing apoptosis, which in turn increases the risk of malignant transformation. P53, Bcl-2, and Fas genes have important roles in apoptosis (5, 6). P53 plays a role in the regulation of the cell cycle, DNA repair and synthesis, and programmed cell death. P53 is a tumor suppressor and attempts to repair DNA damage by arresting the cell in the G1 phase. However, mutant P53 cannot function in the G1 phase, and tumor cells hyperproliferate (7, 8). Bcl-2 protein suppresses and prevents apoptosis. Previous studies have shown that high Bcl-2 expression is correlated with shorter survival and chemotherapy resistance (8, 9). Fas mutations have been found in 20%–30% of various hematological and solid tumors. Fas protein expression has a positive effect on prognosis patients with NHL (10). Various studies have shown that P53, Bcl-2, and Fas protein expression provide valuable information on the treatment and prognosis of NHL (7, 8, 11).

P53 mutations have been detected in more than 50% of tumors in humans. P53 protein expression differs with respect to histological subtypes in gastrointestinal lymphomas (17%–20%), whereas this rate ranges between 60% and 85% in high-grade lymphomas. Different studies have identified that P53 protein expression is an important prognostic factor that affects the treatment response and overall survival in patients with aggressive NHL (12, 13). In our study, we found P53 expression in 61.5% of the patients with primary gastrointestinal DLBCL. Similar to our study, Navaratnam et al. (13) and Hazar et al. (14) found that the rate of complete remission and the five-year overall survival are lower in patients who have P53 protein expression.

Bcl-2 protein expression has been found in 45%–66% of patients with aggressive NHL (15). In our study, we found Bcl-2 protein expression in 67% of the patients. Similar to the results of our study, Sanchez et al. (16) did not identify a significant correlation between Bcl-2 protein expression and complete remission in patients with aggressive NHL. Gascoyne et al. (17) and Kramer et al. (18) found that the five-year overall survival was shorter in patients with aggressive NHL who had Bcl-2 expression. Similarly, we found that overall survival is shorter in patients who had Bcl-2 expression.

Fas protein expression has been found in 40%–75% of patients with aggressive NHL (19, 20). In the present study, we found Fas protein expression in 72% of the patients. Eser et al. (21) and Chatzitolios et al. (22) found that Fas protein expression is a good prognostic factor that affects complete remission and overall survival in patients with NHL. However, we did not find a significant correlation between Fas protein expression and complete remission, total survival, and other prognostic factors.

Sanchez et al. (16) and Krugmann et al. (23) found that overall survival was lower in patients with P53 protein expression and advanced Ann Arbor stage as a result of the univariate analysis. However, the authors did not find any correlation between Bcl-2 protein expression and overall survival. In the current study, the univariate analysis showed that the overall survival rate was significantly lower in patients with high Ann Arbor stage (stage III or IV), two or more extranodal involvements, high IPI score (high-moderate or high), and P53 and Bcl-2 protein expression ($p<0.05$).

Pagnano et al. (24) found that P53 expression is an independent prognostic factor for overall survival. Similarly, our multivariate analysis showed that P53 is an independent prognostic factor for overall survival.

The present study revealed that the complete remission rate was lower in female patients with poor performance status, high Ann Arbor stage (stage III or IV), high LDH level, B symptoms, two or more extranodal involvements, high IPI score (high-moderate or high), bone marrow involvement, and P53 protein expression. Sanchez et al. (16) found that complete remission is lower in patients with high Ann Arbor stage, high LDH level, and high IPI score (high-moderate or high). Similarly, Pagnano et al. (24) found that complete remission is lower in aggressive NHL patients with high Ann Arbor stage (stage III or IV).

CONCLUSION

Primary gastrointestinal DLBCL and P53 and Bcl-2 protein expression have a negative effect on prognosis and overall survival. However, Fas protein expression has no effect on prognosis and survival. Complete remission is lower in female patients with poor performance status, high Ann-Arbor stage (stage III or IV), high LDH levels, B symptoms, two or more extranodal involvements, high IPI score (high-moderate or high), bone marrow involvement, and P53 protein expression. Patients with P53 and Bcl-2 expression should be considered to have a high risk from the beginning, and these patients should undergo aggressive treatments.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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