Araştırma Makalesi

Ege Tıp Dergisi 45 (1) : 5 -11, 2006

MITOTIC ACTIVITY, P53 AND BCL-2 PROTEIN IMMUNOREACTIVITY DIFFERENCE BETWEEN MORPHOLOGICAL VARIANTS AND SUBTYPES OF DIFFUSE LARGE B CELL LYMPHOMA

DİFFÜZ BÜYÜK B HÜCRELİ LENFOMA MORFOLOJİK VARYANT VE ALTGRUPLARINDA MİTOTİK AKTİVİTE, P53 VE BCL-2 PROTEİN iMMUNREAKTİVİTE FARKLILIĞI

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Key Words : morphological variants and subtypes of diffuse large B cell lymphoma, p53, bcl-2, mitosis

Anahtar Sözcükler : Diffüz büyük B hücreli lenfoma morfolojik varyant ve alt grup, p53, bcl-2, mitoz

SUMMARY

The aim of this study is to assess the antiapoptotic bcl-2 gene protein and p53 tumor supressor gene protein immunoreactivity difference between the morphological variants and subtypes of diffuse large B cell lymphoma (DLBCL) and the relationship between the aforementioned proteins, mitotic activity and survival.

In this retrospective study, we examined the pathologic material which were diagnosed as non-Hodkin lymphoma (NHL) from January 1995 to December 2001 in our pathology department. Among these lymphomas, 123 cases were diagnosed as DLBCL. Of 123 cases, we were able to analyze the morphological variants and subtype, mitotic activity and the expression of p53 and bcl-2 proteins in only 53 cases of which in 31 cases clinical data were available. We examined the relation of aforementioned parameters and survival of these 31 cases.

The diagnosis of DLBCL was established according to the criteria referred in World Health Organization (WHO) classification. Of 53 cases, 22 (41.5%) were centroblastic, 13 (24.5%) anaplastic, 7 (13.3%) immunoblastic, 6 (11.3%) T cell rich B cell lymphoma (TCRBCL) and 5 (9.4%) primary mediastinal B cell lymphoma (PMBCL). We had no cases diagnosed as plasmablastic lymphoma and lymphomatoid granulomatosis. We found some relationships between the morphological variants and subtype of DLBCL and mitotic activity, the expression of p53 and bcl-2 proteins: The expression of p53 protein was observed more frequently in anaplastic lymphomas compared to TCRBCL (92.3% versus 16.7%, p< 0.0008). The expression of bcl-2 protein was observed more frequently in immunoblastic lymphoma compared to anaplastic lymphoma (85.7% versus 53.8%, p< 0.001). In centroblastic lymphoma, median mitotic activity was higher and mitotic figures higher than 20 were observed more frequently. In DLBCL, with or without subclassification, no correlation was found between survival and p53, bcl-2 expression and mitotic activity.

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Makalenin geliş tarihi : 13.07.2005 ; kabul tarihi : 22.07.2005

ÖZET

Bu çalışmada, antiapopitotik bcl-2 gen ve p53 tümör supresör gen proteinlerinin, diffüz büyük B hücreli lenfomaların (DBBHL) morfolojik varyantları ve alt gruplarındaki immunreaktivite farklılıklarının değerlendirilmesi ve sözü edilen proteinler ve mitoz sayısı ile olguların sağ kalım süresi arasındaki ilişkinin ortaya konması amaçlanmıştır.

Retrospektif çalışmamızda, ana bilim dalımızda 1995 yılı Ocak ayı ve 2001 yılı Aralık ayı arasında non-Hodgkin lenfoma tanısı almış materyalimiz gözden geçirilmiş ve DBBHL tanısı almış 123 olgu saptanmıştır. Bunlardan yalnızca 53 olgu morfolojik varyant ve alt grup ve mitoz yanı sıra p53 ve bcl-2 immunohistokimyasal özellikleri bakımından değerlendirilmiştir. Elli altı olgunun 31'inin klinik takibi elde edilebilmiş olup, bu olgular yukarıda sözü edilen parametreler yanı sıra sağ kalım süreleri bakımından da değerlendirilmiştir.

53 olgu Dünya Sağlık Örgütü (WHO) sınıflamasındaki kriterlere göre morfolojik varyant ve alt gruplara ayrılmış ve 22'si (%41.5) sentroblastik, 13'ü (%24.5) anaplastik, 7'si (%13.3) immunoblastik, 6'sı (%11.3) T hücreden zengin B hücreli lenfoma (THZBHL) ve 5'i (%9.4) primer mediastinal B hücreli lenfoma olarak değerlendirilmiştir. Plazmablastik lymphoma ve lenfomatoid granülomatozis tanısı alan morfolojik varyantlar saptanmamıştır. P53 ekspresyonu anaplastik lenfomada (%92.3), THZBHL (%16.7) ile karşılaştırıldığında yüksek oranda saptanmıştır ($p < 0.0008$). Bcl-2 ekspresyonu immunoblastik lenfomada (%85.7), anaplastik lenfoma ile karşılaştırıldığında yüksek oranda saptanmıştır. Sentroblastik lenfomada ortalama mitoz oranı ve 20'nin üzerinde mitoz değeri diğer lenfomalara göre yüksek bulunmuştur. Morfolojik varyantlar ve alt gruplara ayrılmaksızın genel olarak DBBHL'lar yanısıra morfolojik varyantlar ve alt gruptaki p53, bcl-2 ve mitoz değerlerinin sağ kalım süresini etkilemediği belirlenmiştir.

INTRODUCTION

DLBCLs are high grade lymphomas which have different morphological variants and subtypes. These lymphomas constitute 40% of adult non- Hodgkin lymphomas and 20% of childhood NHL (1). DLBCLs have an agressive natural history as well as a good response to chemotherapy. 40% of patients die although therapy is administered. Since the response to therapy differ among patients, many studies are performed to determine the prognostic factors. In DLBCL age, gender, presence of systemic symptoms, serum lactate dehydrogenase levels, and the stage are analyzed for prognosis (2-10). Besides the clinical parameters, it has been presumed that expression of tumor suppressor genes and oncogenes are also important in determining the prognosis, biological behaviour of the tumor and new treatment regimens (11-14).

In several studies, p53 (14,15) and bcl-2 (16) protein expression were reported as independent prognostic factors but some studies suggested that these proteins (13,15,17) had no prognostic significance in DLBCL.

The aim of this study is to assess the antiapoptotic bcl-2 gene protein and p53 tumor supression gene protein immunoreactivity difference between the morphological variants and subtypes of DLBCL and the relationship between the aforementioned proteins, mitotic activity and survival.

MATERIALS AND METHODS

Patients

123 cases were diagnosed as DLBCL from 1995 to 2001 in our pathology department. Of these, 53 cases (30 female, 23 male, median age 56±16) whose formalin fixed and paraffin embedded tissue was available for immunophenotypic studies were selected. The hematoxyline eosine and immunohistochemically (CD20, CD79a, CD45RO; CD3, CD15, CD30, CD68, Lysozyme, MPO, TdT) stained sections which belong to these 53 cases were reevaluated. After confirming that morphological characteristics of the cases fullfilled the criteria of WHO classification (18), we analyzed the mitotic activity and the expression of p53 and bcl-2 proteins in the variants and subtype of DLBCL. All the cases were reclassified as centroblastic, anaplastic, immunoblastic, TCRBCL and PMBCL. Clinical data were available in only 31 of the cases. We examined the mentioned parameters and survival in these 31 cases.

Immunohistochemistry

All tissues used in this study were fixed in 10% neutral buffered formalin and embedded in paraffin. 5 µm thick-sections were prepared from formalin fixed, paraffin embedded tissues and mounted on Poly-L-lysine coated slides. The immunohistochemical procedure defined below was performed on these sections.

After overnight incubation of sections at 54°C, the routine deparaffinization and rehydration steps were followed. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide for five minutes and washed in distilled water. For antigen retrieval, sections were incubated in sodium citrate buffer (0.01 mol/L, 6.0 pH) for two 5 minute cycles in a

household microwave oven (600W). After cooling to room temperature, sections were washed twice with distilled water and once with Tris-buffered saline (TBS) and incubated for 30 minutes at room temperature with primary antibodies [anti-p53 (DAKO, code no: M 7001) and bcl-2 (DAKO, code no: N 1587)], followed by sequential 30 minute incubations with biotinylated secondary antibody and peroxidase labelled biotin-streptavidin. Between each step, sections were washed twice in TBS solution for five minutes at room temperature. The final step for localization of the peroxidase deposition was achieved by diaminobenzidine chromogenic reaction, followed by counterstaining with hematoxylin, dehydration and mounting.

Evaluation of Immunohistochemical Reactions

P53 was quantified by determining the number of positive large cells expressing nuclear p53 positivity among the total 1000 large cells within 10 high power (100×objective) microscopic fields. P53 staining was scored from (+) to (+++) as follows; (+) (1% to 10% positive large cells); (++) (11% to 50%); (+++) (> 51% positive large cells). Bcl-2 was quantified by determining the number of positive large cells expressing cytoplasmic bcl-2 positivity among the total 1000 large cells within 10 high power (100×objective) microscopic fields. Bcl-2 staining was scored from (+) to (+++) as in p53 staining.

Evaluation of Mitotic Figures

The number of mitotic figures was assessed as 0-20, 21-50 and higher than 50 per 10 high power field.

Statistical Analysis

Statistical analyses were carried by computer based programme SPSS version 7.0. Univariate analysis and frequence analysis were used. Correlations were searched by Pearson Chi-Square test. p values less than 0.05 was accepted to display the significance of differences.

RESULTS

Of 53 patients, 30 were women and 23 were men with ages ranging between 25 and 90 years (median 56.4). According to WHO classification, of 53 cases, 22 (41.5%) were centroblastic (figure 1), 13 (24.5%) anaplastic (figure 2), 7 (13.3%) immunoblastic (figure 3), 6 (11.3%) TCRBCL (figure 4) and 5 (9.4%) PMBCL (figure 5). No cases were diagnosed as plasmablastic lymphoma and lymphomatoid granulomatosis.

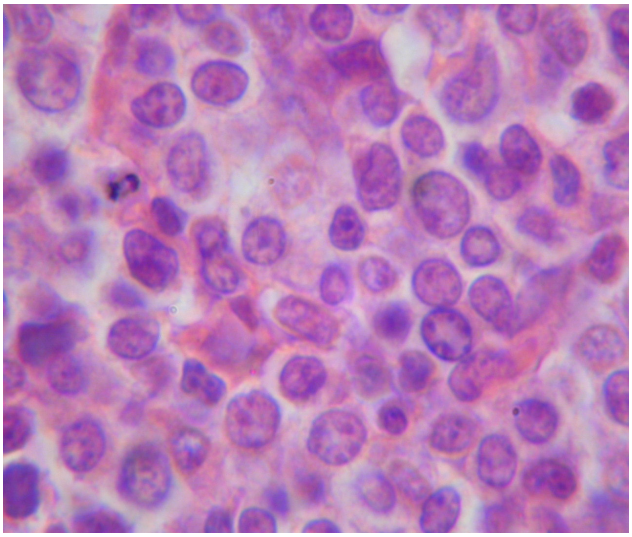

Figure 1. Centroblastic variant of DLBCL (Hemaetoxyline-eosine× 40)

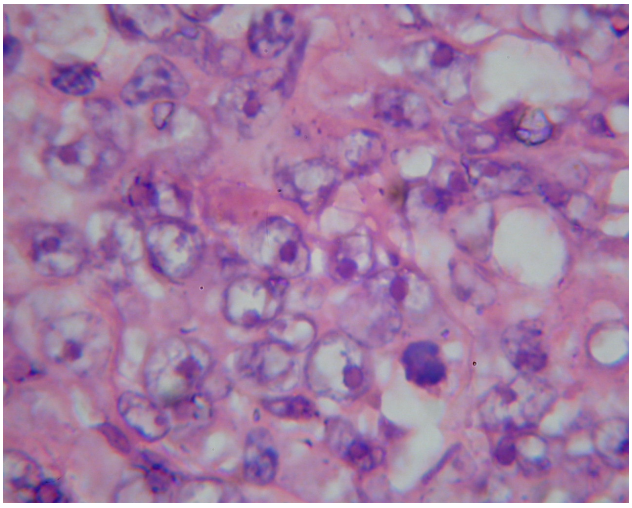

Figure 2. Immunoblastic variant of DLBCL (Hemaetoxyline- eosine × 40)

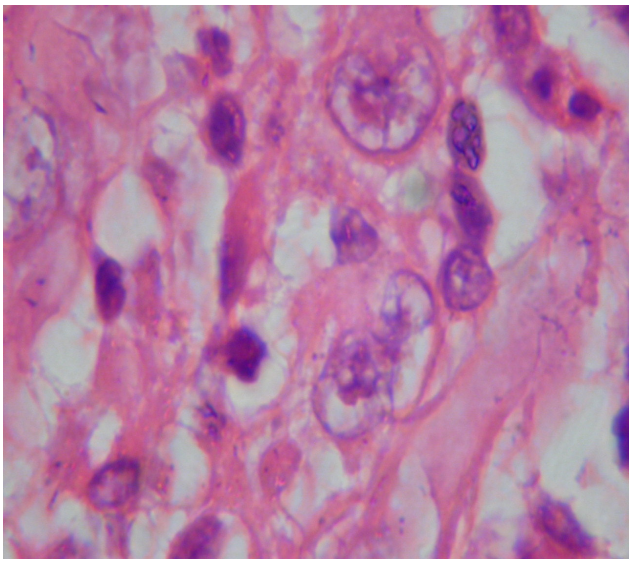

Figure 3. Anaplastic variant of DLBCL (Hemaetoxyline- eosine × 40)

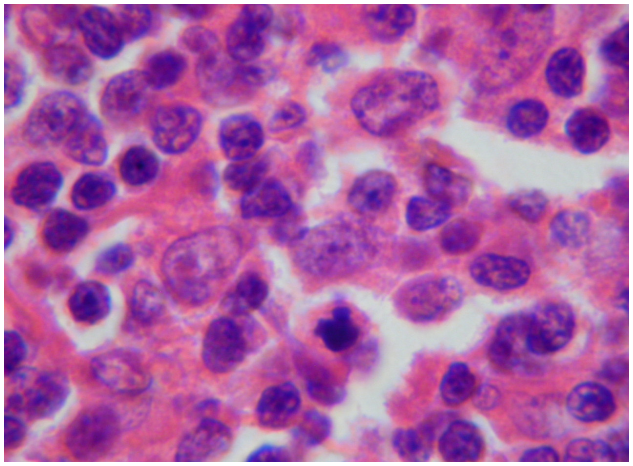

Figure 4 . T cell rich B cell lymphoma (Hemaetoxyline- eosine × 40)

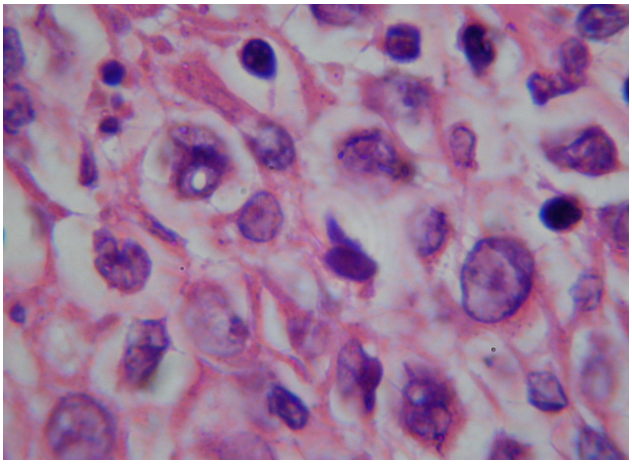

Figure 5. Primary mediastinal B cell lymphoma (Hemaetoxyline- eosine × 40)

Table 1. Mitotic activity in morphological variants and subtype of DLBCL

	0-20	**21-50**	**51-100**	**Total**
Centroblastic	5	8	9	22
Anaplastic	4	5	2	11
Immunoblastic	2	5		**7**
TCRBCL	6			**6**
PMBCL	2			**2**
Total	19	**18**	**11**	**48**

Table 2. p53 immunoreactivity in morphological variants and subtype of DLBCL

	P53 positivity (%)	**Median P53 values (%)**	**+ reactivitity**	**++ reactivitity**	**+++ reactivitity**	**Total**
Centroblastic	40.9	12.3	6 (%23.3)	2 (%9.1)	1 (%4.5)	9
Anaplastic	92.3	30.8	2 (%15.4)	7 (%53.8)	3 (%23.1)	12
Immunoblastic	85.7	42.9	3 (%42.9)		3 (%42.9)	6
TCRBCL	16.7	16.7	1 (%16.7)			1
PMBCL	40	40	2 (%40)			2
Total			14	9	7	30

Tablo 3. bcl-2 immunoreactivity in morphological variant and subtypes of DLBCL

	Bcl-2 positivity(%)	Median Bcl-2 values (%)	+ reactivitity	++ reactivitity	+++ reactivitity	Total
Centroblastic	63.6	31.8		5 (%22.7)	9 (%40.9)	14
Anaplastic	53.8	17.9	1 (%7.7)	3 (%23.1)	3 (%23.1)	7
Immunoblastic	85.7	42.9		3 (%42.9)	3 (%42.9)	6
TCRBCL	66.7	33.4	3 (%50)	1 (16.7)		4
PMBCL	80	80	4 (%80)			4
Total			8	12	15	35

Mitotic activity of the tumors are shown in Table 1. Mitotic figures higher than 50 per 10 high power field were encountered in 9 cases with centroblastic lymphoma (40.9 %) and 2 cases with anaplastic lymphoma (18.2%). Mitotic figures lower than 20 per 10 high power field were encountered in all of the cases of TCRBCL (100 %) and in 5 cases of centroblastic lymphoma (22.7%) ($p< 0.01$). We could not evaluate the mitotic activity in two cases with anaplastic lymphoma and three cases with PMBCL.There was a statistically significant difference in median mitotic activity between centroblastic lymphoma and TCRBCL ($p< 0.009$).

P53 Expression

Among the 53 cases, p53 protein expression was found in 30 (56.6 %) cases. P53 immunoreactivity of the tumors are shown in Table 2. 12 (92.3 %) cases with anaplastic lymphoma and only one case (16.7 %) with TCRBCL had p53 expression. This difference was found to be statistically significant ($p< 0.0008$). There was also a significant difference in cell staining intensity between p53 positive cases . Of 16 cases, (++) and (+++) reactivity was seen in 10 (62 %) cases of anaplastic lymphoma, 3 (19 %) of centroblastic lymphoma and 3 (19 %) of immunoblastic lymphoma ($p< 0.0008$ for both)

Bcl-2 Expression

Among the 53 cases, bcl-2 protein expression was found in 35 (66 %) cases. Bcl-2 immunoreactivity of the tumors are shown in Table 3. Bcl-2 protein expression was observed in 6 (85.7%) cases with immunoblastic lymphoma and 7 (53.8%) cases with anaplastic lymphoma. This difference was found to be statistically significant ($p< 0.001$). Among bcl-2 positive cases, there was a significant difference in cell staining intensity. (+++) reactivity was seen in 9 (60 %) cases of centroblastic lymphoma, 3 (20 %) of anaplastic lymphoma and 3 (20 %) of immunoblastic lymphoma ($p< 0.01$ for both).

Survival

Clinical follow up ranged from 8 to 66 months. Of 31 patients, 10 (32.3 %) died because of lymphoma. The remaining 21 (67.7%) patients survived. The relation between the survival and mitotic activity, p53 and bcl-2 protein expression in DLBCL was examined. No significant relation was observed ($p> 0.05$).

DISCUSSION

DLBCLs are the most common lymphoid neoplasms of adult NHLs which have different morphological variants and subtypes. Although therapy for these NHLs has greatly improved over the last years, approximately 40% of the patients are not cured by chemotherapy regimens. Since DLBCLs form a heterogeneous group in terms of response to treatment and prognosis, several studies are performed to determine the prognostic factors (11-14). In these studies, besides clinical parameters, expression of oncogenes and tumor supressor genes expression were examined by immunohistochemical and molecular techniques.

The aim of this study was to assess the antiapoptotic bcl-2 gene protein and p53 tumor supression gene protein immunoreactivity difference between the morphological variants and subtypes of DLBCL and the relationship between the aforementioned proteins, mitotic activity and survival.

Mitotic figures were 0-20 in 19 (38.8 %) cases, 21-50 in 18 (37.5 %) cases and higher than 51 in 11 (22.4%) cases. Median mitotic activity in centroblastic lymphoma was higher than TCRBCL ($p< 0.009$). 100 % of the patients with TCRBCL have mitotic figures lower than 20 per 10 high power field, but this percentage was only 22.7% in

the cases with centroblastic lymphoma (p< 0.01). Furthermore mitotic figures higher than 50 per 10 high power field were encountered in 40.9% of centroblastic lymphoma and only 18.2% of anaplastic lymphoma (p< 0.01). When we reviewed the literature, we could not fınd any study comparing the mitotic activity in morphological variants and subtype of DLBCL.

In our study, no significant relation was observed between survival and the mitotic activity in morphological variants and subtype. In several published studies, there were conflicting results. In the study by Vago and colleagues (19), a significant relation was found between high mitotic activity and aggressive course. However in the others, no significant relation was observed between these parameters (11,20).

We found statistically significant p53 protein expression differences between anaplastic lymphomas and TCRBCL (p< 0.0008). P53 positivity was seen in 12 (92.3 %) of 13 cases with anaplastic lymphoma whereas in TCRBCL p53 positivity was present only in 1 of the 6 cases (16.7%).

We found statistically significant bcl-2 protein expression differences between immunoblastic and anaplastic lymphomas (p< 0.001). Bcl-2 positivity was observed in 85.7 % of patients with immunoblastic lymphoma and 53.8% of patients with anaplastic lymphoma. In the literature, there were studies which examined the p53 or bcl-2 protein expression especially in TCRBCL and PMBCL cases (21-23). On the other hand we could not fınd any study comparing the differences in p53 and bcl-2 protein expression in all morphological variants and subtypes of DLBCL

In the literature there were many studies with conflicting results examining the relation between survival and p53 and bcl-2 protein expression in DLBCL without subclassification (24-28). In the study of 119 cases by Piris and colleagues, p53 immunoreactivity was found to be an independent factor in survival (24). They found shorter survival for patients with p53 protein expression. They also found that the patients with coexpression of bcl-2 and p53 protein demonstrated more aggressive prognosis than the patients with only p53 expression. But in this study we could not fınd any relation between coexpression of bcl-2 and p53 protein and prognosis. Koduru et al (14) and Zhang et al (29) also found shorter survival for patients with p53 protein expression in DLBCL. On the contrary, no significant relation was found between p53 protein expression and survival in other studies (13, 28, 30).

Sanchez (30), Hermine (31) and Gascoyne et al (32) observed a relation between bcl-2 protein expression and survival in DLBCL and they found that bcl-2 protein expression was a poor prognostic factor. There are also some studies suggesting no significant relation between bcl-2 protein expression and survival (24, 26, 28, 33). In this study, significant difference between survival and the expression of p53 and bcl-2 in DLBCL patients was not found. This finding may be related to the limited number of our cases.

In our study, we found statistically significant differences in mitotic activity, p53 and bcl-2 protein expression between some morphological variants and subtype of DLBCL. However no significant relation was observed between survival and mitotic activity, p53 and bcl-2 protein expression. The histological diagnosis is quite difficult in some morphological variants of DLBCL, the behaviour and treatment responses of which are different. Thus assesment of differences in mitotic activity , oncogene and tumor suppressor gene expression of these tumors can be usefull for routine histology.

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