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Correlation of Nm23 and P27 Expression in Nodal Diffuse Large B Cell Lymphomas with Clinicopathologic Parameters and the Effect on Prognosis

ORIGINAL INVESTIGATION

Yelda Dere[1], Aylin Orgen Çallı[1], İnci Alacacıoğlu[2], Betül Bolat Küçükzeybek[1], Sadi Bener[1], Bahriye Payzın[2]

ABSTRACT

Objective: Diffuse large B cell lymphoma (DLBCL) is the most common type of adult lymphoma. No certain histopathological parameter was found, except proliferation index, for predicting prognosis. In hematological malignancies, the prognostic effect of Nm23 expression, which shows metastatic potential in solid tumors, has been researched by a few studies. Loss of p27, a negative regulator of the cell cycle, indicates aggressiveness; however, some studies showed that high expression is relevant to poor prognosis. Here, we aimed to investigate whether they can be used as prognostic markers in DLBCLs.

Materials and Methods: Forty-two cases were included in the study. Demographic data were collected from hospital records. Manual immunohistochemical staining procedure was performed on one paraffin block of every case. Intensity and percentage of staining were grouped as positive and negative.

Results: From 25 males and 17 female patients with a mean age of 62.9, 28 cases (66.7%) were Nm23-positive, and 13 (30.9%) were p27-positive. International Prognostic Index (IPI) score was relevant to prognosis. No significant relationship was found between prognosis and the expression of these two markers.

Conclusion: Advanced age, high IPI score, and high serum lactate dehydrogenase (LDH) level were considered poor prognostic factors in DLBCLs. Larger series are needed for using Nm23 and p27 in predicting prognosis because of different results in the literature.

Key words: Lymphoma; B-Cell, NM23 nucleoside diphosphate kinases, cyclin-dependent kinase inhibitor p27, prognosis

INTRODUCTION

Diffuse large B cell lymphoma (DLBCL), which is the most common subtype of lymphoma seen in adults, is generally located in lymph nodes. DLBCLs are a heterogeneous group of lymphomas with different histological features, genetic abnormalities, and clinical outcomes (1). Although DLBCLs are accepted as aggressive lymphomas, 40%-50% of the patients seem to have favorable outcomes (2, 3). Because of these differences, many different clinical parameters are being searched to anticipate the prognosis, such as age, sex, extranodal involvement, and International Prognostic Index (IPI) scoring system (1). Pathologically, there is no certain parameter that has been found to affect the prognosis except proliferation index. In this study, we aimed to show whether immunohistochemical stains of Nm23 and p27 can be used as pathologic parameters to predict the prognosis of DLBCL.

MATERIALS and METHODS

Forty-two cases, diagnosed as DLBCL in Izmir Ataturk Training and Research Hospital Pathology Department between 2005 and 2010, were included in this study. We obtained informed consent from patients and certification from the Dokuz Eylul University Ethical Committee about the relevance of the study.

Demographic features were collected from the automated data system of our hospital (PROBEL HBYS- ORACLE Data Guard, v.1.49) and the records of the Hematology Clinic. Only nodal DLBCL cases were included in our study. Age, sex, date of diagnosis, stage at diagnosis, type of treatment, response to treatment, and total time of follow-up were recorded. Some of the patients were treated in a different institution; so, those patients were called to learn about where they had been treated, and then we called the doctors of their new institutions to find out the latest situation of the disease, what chemotherapy was used, and how the response to therapy was. Cases were graded with the Ann-Arbor grading system and divided into two groups as low risk (score: 0-2) and high risk (score: 3-5) by using the IPI scoring system. Serum lactate dehydrogenase (LDH) levels were grouped as low (<300 U/L) and high (>300 U/L). The treatment procedures that were performed on our cases were chemotherapy or radiotherapy only; both chemotherapy and radiotherapy; both chemotherapy and surgery; and surgery, chemotherapy, and radiotherapy. All of the patients were treated with R-CHOP (rituximab 375 mg/m^2/day, cyclophosphamide

[1]Department of Pathology, İzmir Katip Çelebi University Education and Research Hospital, İzmir, Turkey

[2] Clinic of Hematology, İzmir Katip Çelebi University Education and Research Hospital, İzmir, Turkey

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Correspondance
Yelda Dere MD,
Department of Pathology,
İzmir Katip Çelebi University
Education and Research
Hospital, 35100, İzmir, Turkey
Phone: +90 505 465 31 98
e.mail:
yeldamorgul@gmail.com

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750 mg/m^2/day, doxorubicin 50 mg/m^2/day, vincristine 1.4 mg/m^2/day, methyl-prednisolone 100 mg/day) on a 21-day cycle. According to Cheson criteria, patients were grouped as full remission, partial remission, minimal response, resistant to treatment, and still on treatment.

All hematoxylin-eosin slides were observed by two different pathologists independently, and one paraffin block for each patient was selected for immunohistochemistry. The primary antibodies Nm23-H1 (NDPK-A) (*clone 37.6-Novocastra*) and P27/Kip1 Ab-1 (*clone 256-R7, DCS-72-F6) (Neo Markers*) were used for staining. All immunohistochemistry processes were handled manually, antigen retrieval step was achieved by a microwave oven, and a biotin-streptavidin detection system was used. Breast carcinoma and normal tonsil tissue sections were used as positive controls for Nm23 and p27, respectively. The optimal titer for Nm23 was 1/50 and 1/100 for p27.

Nuclear staining for p27 and cytoplasmic staining for Nm23 were accepted as positive. Intensity and percentage of staining were determined and grouped as:

Strength of staining was scored as:

No staining = 0
Mild staining = 1
Moderate staining = 2
Strong staining = 3 (Figure 1-2)

Diffusiveness of staining was scored as 0%-25% = 0, 26%-50% = 1, 51%-75% = 2 and 76%-100% = 3. Two scores (for intensity and diffusiveness) of each case were added, and one final score was gained. According to these final scores, two main categories were identified: negative expression = final score between 0 and 3, and positive expression = final score between 4 and 6.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) 19 software was used for statistical analysis. In parametric tests, independent t-test and Spearman's rho test were used for normally distributed data, and chi-square, continuity correction, and Fisher's exact tests were used for categorical data. A P value less than 0.05 was considered significant. Survival curves were drawn according to the Kaplan-Meier method.

RESULTS

Forty-two patients with a mean age of 62.9, diagnosed as nodal diffuse large B cell lymphoma, were included in this study. Male patients were 59.5% and female patients were 40.5% of the cases, with an M/F ratio of 1.4/1; 23.8% of all cases were stage I, 26.2% were stage II, 16.7% were stage III, and 33.3% were stage IV. In the statistical analysis, cases were rearranged in two groups as low-stage (stage I and II) and high-stage (stage III and IV). Patients were treated by chemotherapy (66.6%), chemoradiotherapy (31%), and chemotherapy + surgery (2.4%).

Patients were followed up between 1 to 74 months with a mean of 26.7. When we looked upon the effect of treatment, 78.5% of cases were in full remission, 7.2% were in partial remission, and 9.5% were resistant to therapy. Two cases (4.8%) were still on treatment at the time of the study. In total, 73.8% of the cases were alive whilst 26.2% were dead.

Among many clinical parameters, age, sex, stage, type of treatment, serum LDH level, and IPI score were selected to observe the effect on prognosis.

Patients with an LDH level greater than 300 U/L were accepted as high level (26.2%), while 73.8% of the cases had an LDH lower level than 300 U/L. None of these parameters showed a statistically significant relationship with prognosis.

According to IPI score, 16 of the patients (38%) had low, 15 (35.7%) had low-intermediate, 7 (16.7%) had high-intermediate, and 4 (9.6%) had high IPI scores. In the statistical analysis, IPI scores were rearranged into two groups as low IPI score (low and low-intermediate) and high IPI score (high-intermediate and high). We observed a statistically significant relationship between prognosis and IPI score (p: 0.003).

Immunohistochemical staining results are given in Table 1, showing the correlation to survival rates.

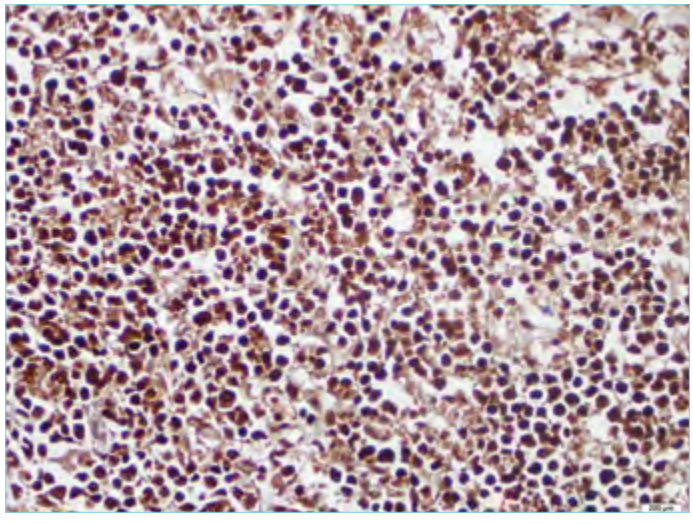

Figure 1. **Strong nuclear staining of p27, DAB, X400**

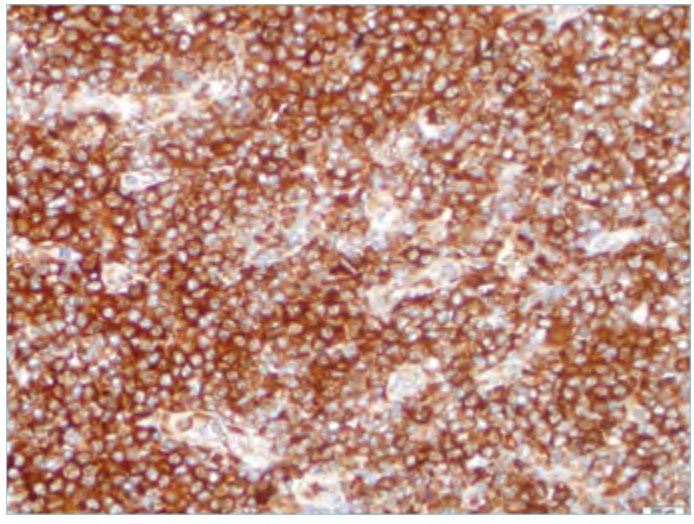

Figure 2. **Strong cytoplasmic staining of Nm23, DAB, X400**

Table 1. Correlation between immunohistochemical staining results and survival rates

Survival		Alive		Dead			
		Number of cases	Percentage	Number of cases	Percentage	Total	p value
Immunohistochemical Marker	P27 (+)	9	21.4%	4	9.5%	42 (100%)	0.713
	P27 (-)	22	52.4%	7	16.7%		
	Nm23 (+)	20	47.6%	8	19.1%	42 (100%)	0.723
	Nm23 (-)	11	26.2%	3	7.1%		
	P27 (+)/Nm23 (-)	3	7.1%	0	0%	42 (100%)	0.554
	P27 (-)/Nm23 (+)	14	33.4%	4	9.5%		0.731
	P27 (+)/Nm23 (+)	7	16.7%	3	7.1%		1
	P27 (-)/Nm23 (-)	7	16.7%	4	9.5%		0.437

No significant relationship was found between p27 and Nm23 and prognosis (p: 0.713 and 1, respectively).

DISCUSSION

Diffuse large B cell lymphoma (DLBCL) has many clinical and histopathological differences and generates the largest group of lymphomas. Beyond the aggressiveness, 40%-50% of the cases respond to therapy (2, 3). In recent years, with new target-based chemotherapy and radiotherapy, the survival rates can reach up to 90% (2).

Diffuse large B cell lymphomas are a heterogeneous group of lymphomas with differences in clinical and histological features, genetic abnormalities, response to treatment, and survey. In addition to these differences, genetic and molecular alterations have been valuable in predicting the prognosis and the risk of the patients (1).

Diffuse large B cell lymphomas are generally seen on in the sixth decades, similar to our result of 62.9 (2, 4, 5). DLBCLs show a slight male predominance with a ratio of 1.2 (6). In our study, concordant with the literature, among all 42 non-Hodgkin lymphoma (NHL) patients, 59.5% were male and 40.5% were female, with a male/female ratio of 1.4 (5, 6).

The IPI scoring system is one of the most commonly used prognostic markers in NHL and consists of patient age, performance status, serum LDH level, Ann Arbor stage, and extranodal involvement of the disease (7, 8). Because all these parameters were shown to be effective on the prognosis, IPI scoring was calculated for every patient in our study.

Many studies showed that high IPI score is relevant with low survival rates (5, 7, 9). Similar to this, Uzurov-Dinic et al. (10) found that patients with low and low-intermediate IPI scores had a better prognosis. Concordant with the literature, a statistically significant relationship was found between IPI score and prognosis in our study (p: 0.003).

Although IPI score consists of many clinical parameters, we also observed them one by one whether any prognostic differences occurred. Patients over 60 years tend to have lower survival rates compared to younger cases (7, 11). Two different studies of Zhang et al. (12) and Lopez-Guillermo et al. (5) showed that advanced age is one of most effective factors in prognosis and disease-free survival. In our study, 21.4% of patients over 60 years were dead, but no significant relationship was found between age and prognosis (p: 0.08). Many studies stated that advanced stage (stage III-IV) is relevant to low survival rates (5, 13). Similar to these results, advanced cases showed poor prognosis in our study, but this was not statistically significant (p: 0.079). Lopez-Guillermo et al. (5) found that patients with high serum LDH level had statistically significantly low survival rates, but in our study, no significant relationship was found between these two parameters (p: 0.410) (5).

Many different parameters are included in the IPI scoring system except sex. In Tomita et al. (14), male sex, extensive extranodal involvement, and coexisting B symptoms were evaluated as poor prognostic factors (14). Among our patients, 28.6% of women and 45.6% of men were alive. According to these results, no significant relationship was found between sex and survival (p: 0.733). In order to obtain certain results to prove this relationship, we strongly suggest that large series and at least partially equal number of sex subjects should be included into the newly conducted research.

P27KIP1, located on 12p13, is a cyclin-dependent kinase inhibitor and has an important role in the cell cycle, from G1 to S phase transition (15). P27KIP1 is found to be effective in signal transmission between cells, the cyclic adenosine monophosphate (cAMP) pathway, differentiation of cells, apoptosis, response mechanisms to chemotherapeutics, and drug resistance of solid tumors (16). Decrease in p27 expression was found to be associated with aggressiveness and short survey in many different types of tumors, such as gastric, prostate, cervix carcinomas, and renal tumors (15, 17).

Different results were reported about p27 expression in hematologic malignancies. P27 expression was more commonly seen in nodal lymphomas rather than extranodal lymphomas (18, 19).

Generally, p27/KIP1 expression shows an inverse relationship with cell proliferation in lymphomas (19, 20). In many studies, high-grade NHLs showed low p27 expression, concordant with low survival rates, whereas low-grade NHLs showed higher p27 expression (19).

Low-grade lymphomas, such as follicular lymphoma, marginal zone lymphoma, and chronic lymphocytic lymphoma (CLL)/small lymphocytic lymphoma (SLL), show high p27 expression, mantle cell lymphomas show low p27 expression, and low expression of p27 has been associated with short survey and aggressive behavior (21). In DLBCL and Burkitt lymphomas, the relationship between low p27 expression and poor prognosis was emphasized (19, 21, 22).

In contrast to this, some studies stated that high-grade lymphomas showed higher p27 expression (19, 20). P27 expression in lymphomas with high proliferation index, such as DLBCL, Burkitt lymphoma, and anaplastic large cell lymphoma, is stated to be associated with low survival rates by different authors (22, 23).

In the study of Sato et al. (24), p27 expression was determined to be higher in mucosa-associated lymphoid tissue (MALT) lymphomas and lower in DLBCLs, and they pointed out that decrease in p27 expression should alert to one to transformation to DLBCL. But Lim et al. (21) and Martinez et al. (25) suggested that intermediate-grade lymphomas, such as mantle cell lymphoma, showed lower p27 expression than high-grade ones. Similar to this result, in the study of Barnouin et al. (22), higher p27 expression was detected in Burkitt lymphoma, one of the high-grade lymphomas.

In our study, 30.9% of our cases showed p27 expression. When this expression was compared to prognosis, 9.5% of dead patients were p27(+) and 16.7% were p27(-). Although p27 expression was thought to have an inverse effect on prognosis, this hypothesis could not be certified statistically (p: 0.713). Also, p27 expression was not statistically relevant to other clinical parameters, such as age, stage, IPI score, and serum LDH level.

The Nm23 gene, known as a metastasis suppressor gene, is located on the q21.3-22 region of human chromosome 17. Nm23 expression has been investigated widely in breast, gastric, ovarian, cervix, and hepatocellular carcinomas and malignant melanoma, and low expression has been found to be associated with high metastatic potential (26).

Niitsu et al. (27) found that Nm23 expression was associated with short survival and could be used as a prognostic marker in Hodgkin lymphoma. Many different studies by the same author about Nm23 expression in NHLs exist in the literature. In one of them, with 108 cases, Nm23 expression was compared to survival rate and found to be as important as serum Nm23 level in prognosis (28). In this study, Nm23 expression was also associated with performance status and B symptoms.

Nm23 expression has been found to reflect high proliferative index and aggressiveness of the lymphoma in the study of Aryee (29). Lee et al. (26) reported that high Nm23 expression was associated with high IPI score and poor prognosis.

In our study, 66.7% of the cases showed Nm23 expression, and neither clinical parameters nor prognosis showed a statistically significant relationship.

CONCLUSION

As a result, in this study, IPI score was found to be the only parameter statistically relevant to prognosis (p: 0.003). According to this result and also similar to the literature, IPI score was thought to be extremely important in predicting the prognosis of DLBCLs.

Nm23 and p27 expression has been detected in DLBCLs, but these markers showed no significant effect on prognosis. All these conflicting results point out the necessity of more studies with a wider number of cases and longer follow-up to ensure the real prognostic importance of these markers.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Dokuz Eylül University Ethical Committee.

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

Peer-review: Externally peer-reviewed.

Authors' Contributions: Conceived and designed the experiments or case: YD, AOÇ. Performed the experiments or case: YD, AOÇ, İA, SB, KBP. Analyzed the data: İA, BBK, SB, KBP. Wrote the paper: YD, AOÇ, İA, BBK. All authors read and approved the final manuscript.

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