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p53, bcl-2, and nm23 Expressions in Serous Ovarian Tumors: Correlation with the Clinical and Histopathological Parameters

Seröz Over Tümörlerinde p53, bcl-2 ve nm23 Ekspresyonu: Klinik ve Histopatolojik Parametreler ile İlişkisi

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ABSTRACT

Objective: We studied p53, bcl-2, and nm23 expressions in serous benign, borderline and malignant tumors of the ovary. Our aim was to determine the association between these expressions and some clinical (patient age, tumor dimension, omenthal involvement, the presence of malignant cells in peritoneal fluid) and histopathological (grade, number of mitosis, nuclear pleomorphism, structural pattern) parameters in malignant serous tumors.

Material and Method: A total of 71 tumors including 29 benign, 14 borderline, and 28 malignant ovarian serous tumors were included in the study. p53, bcl-2, and nm23 immunohistochemical staining was performed on the paraffin blocks. The results were scored as (+), (++) and (+++) according to the extent of staining.

Results: There was no staining for p53 in benign or borderline tumors. p53 was positive in 42.9% of malignant tumors. nm23 expression was revealed as 44.8%, 64.3% and 67.9% in benign, borderline and malignant tumors, respectively. Bcl-2 was positive in only 17.2% of benign, 35.7% of borderline and 25% of malignant tumors.

Conclusion: The p53 positivity detected only in serous carcinomas shows its role in carcinogenesis. p53 was expressed at a significantly higher rate in advanced stage carcinomas (p=0.031). nm23 expression in benign, borderline and malignant tumors was not significantly different. nm23 positivity was higher in advanced stage carcinomas (p=0.032). This suggest that nm23 acts like an oncogene in ovarian carcinomas. There was no significant difference between the groups in terms of bcl-2 expression.

Key Words: Ovary, Neoplasms, Serous neoplasms, p53, bcl-2, nm23

ÖZ

Amaç: Çalışmamızda overin benign, borderline ve malign seröz tümörlerinde immünhistokimyasal olarak p53, bcl-2 ve nm23 ekspresyonun araştırılması, malign tümörlerde bu ekspresyonların evre, yaş, tümör boyutu, omentum tutulumu, periton sıvısında malign hücre varlığı gibi özelliklerle ve bazı histopatolojik parametrelerle (grade, mitoz sayısı, nükleer pleomorfizm, yapısal patern) ilişkisinin belirlenmesi amaçlandı.

Gereç ve Yöntem: Çalışmaya 29 benign, 14 borderline, 28 malign seröz tümör olmak üzere toplam 71 olgu alındı. Parafin blokta p53, bcl-2 ve nm23 ile immünhistokimyasal çalışma yapıldı. Sonuçlar boyanma yaygınlığına göre (+), (++), (+++) şeklinde skorlandı.

Bulgular: Benign ve borderline tümörlerde p53 ile boyanma yoktu. Karsinomlarda ise %42,9 oranında ekspresyon saptandı. nm23 ekspresyonu, benign, borderline ve malign tümörlerde sırası ile %44,8, %64,3 ve %67,9 olarak bulundu. Bcl-2 ekspresyonu benign tümörlerde %17,2, borderline tümörlerde %35,7, karsinomlarda %25 idi.

Sonuç: p53'ün overin sadece seröz karsinomlarında pozitif bulunması karsinogenezde rol aldığını göstermektedir. p53 ileri evre olgularda daha fazla eksprese olmaktadır (p=0,031). Nm23'ün benign, borderline ve malign tümörlerde ekspresyon oranları istatistiksel olarak farklı değildir. Karsinom olgularında ise ileri evrede nm23 pozitifliği anlamlı derecede yüksektir (p=0,032). Bu durum nm23'ün over karsinomlarında bir onkogen gibi davrandığını düşündürmüştür. Bcl-2 ekspresyonu açısından gruplar arasında anlamlı fark yoktur.

Anahtar Sözcükler: Over, Neoplazmlar, Seröz neoplazmlar, p53, bcl-2, nm23

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INTRODUCTION

Ovarian carcinomas make up 4% of cancers seen in females and they are the gynecological cancers that lead to the most deaths (1). They are seen especially in Scandinavian countries among developed countries and are relatively less common in Japan and developing countries (2). The lack of symptoms in the early period leads to most cases being diagnosed at advanced stages.

Serous tumors make up approximately one-fourth of all ovarian tumors. Despite the data obtained regarding pathogenesis, the precursor lesions of ovarian carcinoma are not clearly known. The lack of benign-appearing areas in serous carcinomas indicates that these tumors develop directly from the epithelium without a precursor lesion. Some authors have stated that there may be a rapid adenoma-carcinoma sequence in the development of the tumors and that precursor lesions may not be seen for that reason (3). There is no generally accepted grading system for ovarian carcinomas. The dual grading system suggested by Malpica for serous carcinomas that has been said to overlap with the dualistic carcinogenesis model postulated by Kurman has been discussed recently (4). The most commonly used system is the universal grading system of Shimizu-Silverberg. The growth pattern, number of mitoses and nuclear pleomorphism are scored and graded to increase the inter-observer consistency in this system (5).

The p53 tumor suppressor gene is active at a cell cycle control point and plays a role in providing a response to DNA damage (6,7). Although it is generally accepted that p53 mutations, shown to affect the prognosis in many cancers, are an unfavorable prognostic factors in ovarian carcinoma, there are also reports that they have no effect on the prognosis (8,9). The mean rate of reported p53 positivity is 51% in serous carcinomas and 1% in borderline and benign tumors (10).

The Bcl-2 gene is a proto-oncogene located on the 18th chromosome. The Bcl-2 gene product is an integral membrane protein located in the internal membranes of mitochondria and is named the bcl-2 protein. The Bcl-2 gene plays an important role in programmed cell death (11). Mano et al. have reported a bcl-2 expression of 25% in stage I and 44% in stage 2 cases and postulated that the response to chemotherapy decreased with increased expression (12). Chan states that bcl-2 expression decreases with a shift from benign tumors to malignant tumors (13). Geisler has claimed that the p53 and bcl-2 combination is an independent parameter of survival. Cases that are p53 negative and bcl-2 positive have the best prognosis (14). The p53 mutant protein can inhibit the production of the bcl-2 protein and mRNA. This indicates that the reason for bcl-2 downregulation in malignant tumors may be the inhibitory effect of p53 (15).

nm23 is located on codon 21.3 of the long arm of chromosome 17 in humans. It has first been defined by Steeg in 1988 as the metastasis suppressor gene that codes the nucleotide diphosphate (NDP) kinase protein (16). It is claimed that nm23 suppresses metastasis but its mechanism of action in metastasis suppression has not been clearly demonstrated. It has been reported to correlate with a predicted decreased metastatic potential by taking into account the decreased nm23 expression in breast, gastric, cervical cancers, hepatocellular carcinoma and malignant melanoma and features such as survival, the presence of lymph node metastasis and little differentiation (17). There are articles stating contradicting results on the effect of nm23 on ovarian carcinoma prognosis (18,19).

The aim of our study was to investigate p53, bcl-2 and nm23 expression immunohistochemically in benign, borderline and malignant serous tumors of the ovary and also to determine the association between these "markers" and features such as age, tumor size, stage, omentum involvement and the presence of malignant cells in the peritoneum and some histopathological parameters (grade, number of mitoses, nuclear pleomorphism, structural pattern).

MATERIAL and METHOD

We included a total of 71 serous ovarian tumors diagnosed at the Ankara Numune Training and Research Hospital Department of Pathology in this study. The tumors were grouped as malignant, borderline and benign. Carcinoma cases were evaluated for stage, age, tumor size, grade, histopathological features (structural pattern, number of mitoses, nuclear pleomorphism), omentum involvement and the presence of malignant cells in peritoneal fluid. The Shimizu-Silverberg system was used for tumor grading as follows: 1 point if the dominant growth pattern was glandular, 2 points if papillary and 3 points if solid. The cytological atypia was graded according to severity as 1, 2 or 3. Mitotic figures were counted in 10 high-power fields and 9 or less was given a score of 1, 10-24 2 and 25 or more 3. The sum of these scores were evaluated as tumor grade I if 3 to 5, II if 6 or 7 and III if 8 or 9 in number. The age of the cases and tumor size were determined in benign and borderline tumors.

The paraffin block that best reflected the morphology among the tumor samples was chosen and sections 5 μm thick prepared. The streptavidin-peroxidase method was used

for the immunohistochemical evaluation. The endogenous peroxidase activity was blocked by incubation with hydrogen peroxide for 10-15 minutes following deparaffinization and rehydration. The tissues were treated with primary prediluted monoclonal antibody. The markers used were Ab-5, clone DO-7 (Neomarkers) for p53, Ab-1 (Neomarkers) for nm23 and bcl-2α Ab-1, clone 100/D5 (Neomarkers) for bcl-2. The sections were washed and incubated for 10 minutes in biotinylated goat antipolyvalent conjugate. This procedure was repeated for 10 min with the streptavidin-peroxidase complex. Chromogen was applied and contrast staining was obtained with Mayers hematoxylin. Glycerol was used to cover. The control materials were larynx squamous cell carcinoma for p53, colon adenocarcinoma for nm23 and tonsilla palatina for bcl-2.

We evaluated tumor cell cytoplasmic membrane staining and cytoplasmic staining for bcl-2, nuclear staining for p53, and cytoplasmic staining for nm23 during immunohistochemical evaluation. The grading was (+) for 1-10% staining, (++) for 10-50% staining and (+++) for more than 50% staining. The statistical analysis was performed using these staining results. The two groups were then separated as negative and positive and the statistical difference of the parameters between the groups evaluated.

The data obtained were analyzed with the "Mann-Whitney U" test, ANOVA, Kruskal-Wallis, Chi square, and Spearman's correlation test during statistical analysis. A p value <0.05 was considered statistically significant for all tests.

RESULTS

The distribution of the serous tumors was 29 benign, 14 borderline and 28 malignant. The mean age was 58.6 for serous carcinomas, 50.3 for benign serous tumors and 48.0 for borderline serous tumors. The mean tumor size was 10.2 for serous carcinomas and 17.2 and 15.0 cm for the benign and borderline serous tumors, respectively. Table I summarizes the clinical and histopathological features of serous carcinomas.

p53 expression was not observed in benign or borderline serous tumors (Figure 1). The p53 expression rate in serous carcinomas was 42.9% (p=0.00) (Table II). We found no correlation between tumor stage and p53 staining diffuseness (p=0.765). However, p53 positivity was significantly higher in the advanced stages when the cases were groups as early and late stage (p=0.031). There was no relationship between p53 staining diffuseness and grade in carcinomas (p=0.169).

The mean age was 55.5 and 62.7 for the cases with and without p53 expression, respectively. There was no difference

Table I: Clinical and histopathological features in serous carcinoma cases

		n	%
Bilaterality	unilateral	5	17.9
	bilateral	23	82.1
Omentum involvement	-	6	21.4
	+	22	78.6
Malignant cells in peritoneal fluid	-	3	10.7
	+	25	89.3
Stage	I	3	10.7
	II	3	10.7
	III	11	39.3
	IV	11	39.3
Grade	I	9	32.1
	II	12	42.9
	III	7	25.0
Tumor growth pattern	glandular	9	32.1
	papillary	12	42.9
	solid	7	25.0
Pleomorphism	mild	4	14.3
	moderate	13	46.4
	marked	11	39.3
Number of mitoses (10 HPF)	≤9	10	35.7
	10-24	10	35.7
	≥25	8	28.6
	Total	28	100.0

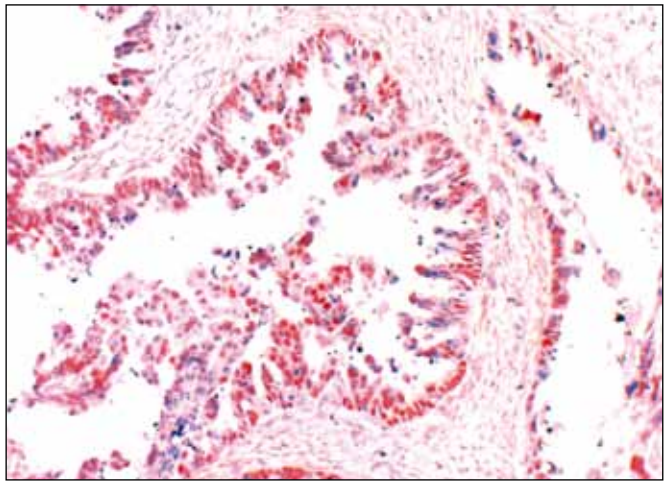

Figure 1: Serous carcinoma (p53; x100).

Table II: p53, bcl-2 and nm23 staining results in serous tumors

		p53				bcl-2		nm23				Total
		-	+	++	+++	-	+	-	+	++	+++	
Serous carcinoma	n	16	10	1	1	21	7	9	15	3	1	28
	%	57.1	35.7	3.6	3.6	75.0	25.0	32.1	53.6	10.7	3.6	100.0
Borderline serous tumor	n	14	0	0	0	9	5	5	3	2	4	14
	%	100.0	0	0	0	64.3	35.7	35.7	21.4	14.3	28.6	100.0
Benign serous tumor	n	29	0	0	0	24	5	16	10	1	2	29
	%	100.0	0	0	0	82.8	17.2	55.2	34.5	3.4	6.9	100.0

between the groups with and without p53 expression for age, tumor size, bilaterality, omentum involvement, the presence of malignant cells in the peritoneal fluid and histopathological parameters (tumor development pattern, pleomorphism, number of mitoses).

Bcl-2 expression was 17.2% in benign serous tumors, 35.7% in borderline serous tumors and 25% in serous carcinomas (Figure 2,3). We found no (++) or (+++) staining in the cases (Table II). The bcl-2 expression was 1 to 10% in all (+) cases. Five of the 7 bcl-2 positive serous carcinoma cases were stage IV. Advanced stages had higher rates of bcl-2 expression but this was not statistically significant (p=0.168). The bcl-2 expression rate was 33.3% in grade I and 33.3% in grade II cases while we found no staining in grade III cases. There was no relationship between bcl-2 expression and grade in carcinomas. Omentum involvement and malignant cells in the peritoneum were present in 7 cases with bcl-2 expression but we found no significant difference between this group and the group with no bcl-2 expression (p=0.288). There was no difference between the bcl-2 negative and positive groups for age, tumor size, bilaterality or histopathological parameters (tumor growth pattern, pleomorphism, number of mitoses).

The nm23 expression frequency was 44.8%, 64.3%, and 67.9% respectively in benign, borderline and malignant serous tumors (Figure 4,5). The difference between the groups was not statistically significant (p=0.192). However, nm23 expression was significantly higher in borderline tumors than benign tumors in terms of staining diffuseness (p=0.012). Although staining diffuseness was thought to be increased in advanced stage cases in carcinomas, we found no statistically significant difference (p=0.116). Evaluating nm23 positivity showed that advanced stage cases expressed nm23 at a statistically significantly higher rate (p=0.032). The nm23 expression rate was 66.7%, 58.3% and 85.7% for Grade I, II and III tumors, respectively. We found no significant relationship between nm23 and grade (p=0.502). The nm23 expression was significantly higher in serous carcinoma cases with omentum involvement

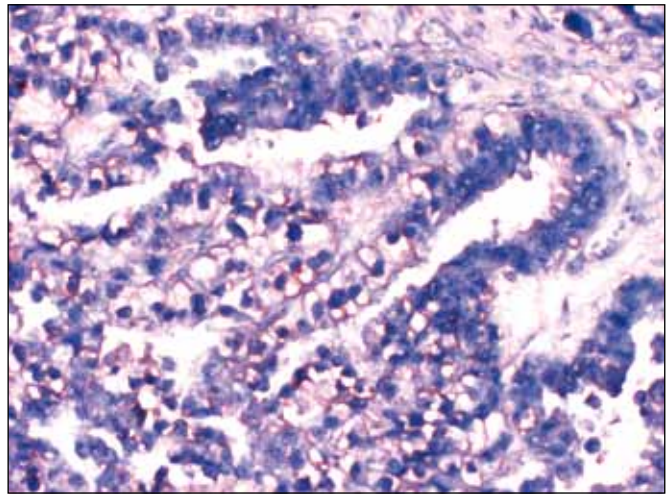

Figure 2: Grade III serous carcinoma (bcl-2; x400).

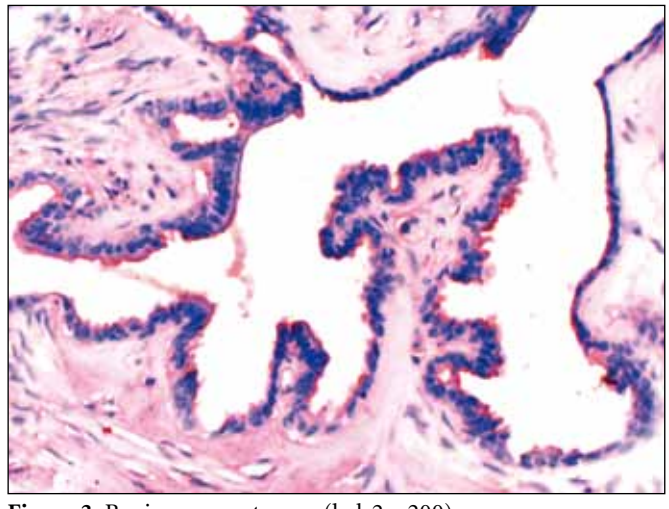

Figure 3: Benign serous tumor (bcl-2; x200).

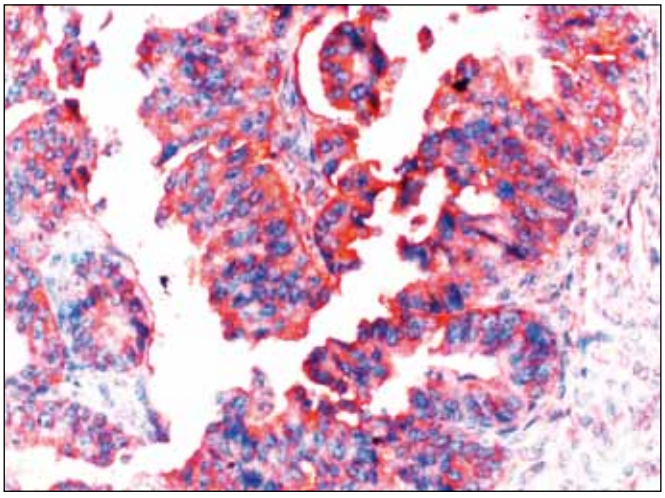

Figure 4: Grade I serous carcinoma (nm23; x400).

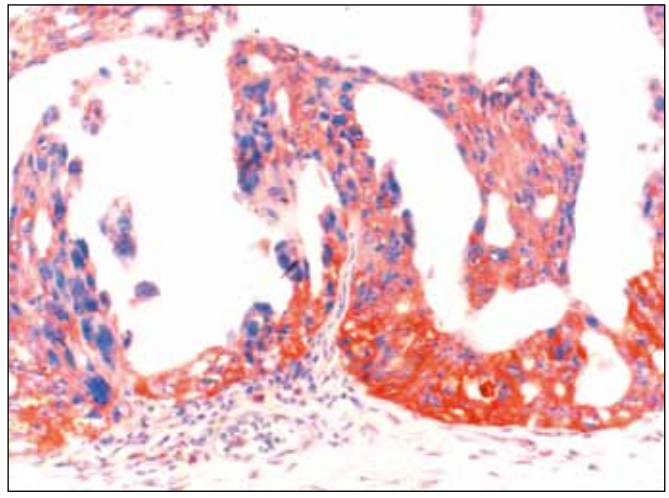

Figure 5: Borderline serous tumor (nm23; x200).

(p=0.041). There was no difference for age, tumor size, bilaterality, the presence of malignant cells in the peritoneal fluid and histopathological parameters (tumor development pattern, pleomorphism, number of mitoses) between the (-), (+), (++), and (+++) nm23 expression groups in serous carcinomas. Classifying into two groups as nm23-negative and -positive showed less nm23 positivity in cases with a glandular growth pattern than those with a papillary and solid growth pattern (p=0.01).

We found a positive correlation between nm23 and bcl-2 in serous carcinomas (p=0.04). nm23 expression was significantly more frequent in cases with Bcl-2 expression. We found no correlation between p53 and bcl-2 (p=0.86). We thought there was an inverse correlation between p53 and nm23 but could not demonstrate this on statistical analysis (p=0.579).

DISCUSSION

Ovarian carcinomas make up 90% of malignant tumors of the ovary (1). The global 5-year survival of ovarian carcinoma cases ranges from 32% to 46%. The generally accepted independent prognostic factor for ovarian carcinomas is the FIGO stage (20). The serous carcinomas in our study were diagnosed at stage I in 10.7%, stage II in 10.7%, stage III in 39.3% and stage IV in 39.3%. This means that approximately one-fifth of the cases were early stage (stage I and II) while four-fifths were late stage (stage III and IV).

p53 is the most common genetic change in cancer. Kmet et al. has reported p53 overexpression at a mean rate of 51% in a total of 6839 ovarian carcinoma cases in their review of studies in the literature (10). The results obtained with immunohistochemical methods are known to change according to the monoclonal antibody used. The most common antibodies used for p53 are DO-7 and PAb-1801 monoclonal antibodies. These antibodies detect both "wild" type and mutant p53 protein. We used the p53 DO-7 monoclonal antibody in our study and the results signify the p53 overexpression in cases with and without p53 mutations (eg. DNA repair process). It is necessary to use polymerase chain reaction (PCR) and SSCP (single-strand conformation polymorphism) techniques to specifically show a p53 mutation. Results obtained with these methods show a mutation rate of 51% in serous carcinomas (10). Schuijer and Berb state a p53 overexpression rate of 59% and mutation rate of 58% in serous carcinomas (21). The p53 expression results obtained with the immunohistochemical technique may not fully correlate with the results obtained through specific mutation analyses but the correlation between overexpression and mutation is statistically significant (22). There is 68% concordance between p53 mutation analyses and the immunohistochemistry results (23).

We found a p53 overexpression rate of 42.9% in our study. The literature reports positive results of 48.5% to 69% in studies using the p53 DO-7 antibody (8,13,22,23). Our results are lower than the literature results on average. Many factors influence p53 study results. The different tissues used in different studies can give different results. The p53 antigen is reported to be better presented in fresh tissue studied by frozen section (22). The different enzymes used for staining and the microwave procedure can also have a marked effect on the staining results. Another reason for the different reported results is the different 'cut off' values used in the studies.

Comparing the stage with p53 expression, Skirnisdottir et al. reported positivity rates of 17.4% in grade I, 47.8% in grade II and 34.8% in grade III cases in 106 ovarian carcinoma patients. High-grade tumors are reported to show significantly higher p53 expression than low-grade tumors (24). We found more p53 expression in grade II tumors in our study while grade III tumors showed less p53 expression than expected. We believe this is due to the low number of cases. The difference between the groups was not statistically significant (p= 0.169).

Werness has reported that p53 expression does not show a correlation with clinicopathological parameters or the prognosis and that cases with p53 expression are older than those without, and that these tumors are of the serous histological subtype and high-grade tumors (8). The mean age was 55.5 in those with p53 expression and 62.7 in those without in our study. The difference was not significant (p=0.413).

A study on p53 expression in the normal ovary and ovarian tumors has found p53 expression in 43% of immunohistochemicallly normal ovary tissue, 18% in benign epithelial tumors, 19% in borderline tumors and 53% in carcinomas. Mutation analysis with PCR and SSCP in the same cases showed a p53 mutation rate of 55% (13). Finding a higher rate of p53 in serous carcinomas is expected. The p53 expression distribution shows a larger number of (+) results. However, Inoue has reported larger numbers of (++) and (+++) staining in a similar study (25).

A bcl-2 expression rate of 19-33% has been reported in ovarian carcinomas (9,13,14,24,26). Chan has reported the bcl-2 expression positivity rate in normal ovary tissue and benign, borderline and malignant ovarian tumors as 79%, 100%, 78% and 33%, respectively. A significant decrease in bcl-2 expression from benign tumor to malignant tumors was reported (13). We found no difference in bcl-2 expression between benign, borderline and malignant serous tumors in our study. Seven bcl-2 positive serous carcinomas out of 28 cases were distributed as stage 2 in 1 case, and stage 3 or 4 in 6 cases. The bcl-2 positivity was higher in advanced stage cases but grade evaluation showed that bcl-2 positive cases were grade 1 and 2. We believe this discordance may be due to the low number of cases.

Studies on bcl-2 in ovarian carcinoma usually include p53 and evaluate the relationship with prognosis. There are only a few studies comparing histopathological and clinical parameters with bcl-2 expression. Sagarra et al. reports a bcl-2 expression rate of 8% in grade I carcinomas, 21% in grade II carcinomas and 24% in grade III carcinomas with bcl-2 positivity increasing as the grade increases (9). Chan et al. and Diebold et al. state that the bcl-2 expression decreases as the grade increases with statistical significance (13,26). We found a bcl-2 expression rate of 33.3% in grade I cases, 33.3% in grade II cases and no staining in grade III cases. We did not find a significant difference between the histopathological and clinical parameters and bcl-2 expression on statistical studies.

Chan et al. has reported the lack or low levels of bcl-2 expression in an ovarian cancer with diffuse p53 expression. It was also reported that p53 was not found in tumors with high levels of bcl-2 expression (13). Our study results indicate an inverse correlation between p53 and bcl-2 but statistical evaluation did not demonstrate the correlation.

The role of nm23 in gynecological cancers is not clear. The nm23 expression has been reported to decrease in cases with lymph node metastasis and tumors with an aggressive course (26,27). Schneider et al. has found nm23 expression in 12 of 26 serous carcinoma cases of which 9 were at an advanced stage and postulated that nm23 acts like an oncogene in addition to its potential antimetastatic effect and that it is expressed at a significantly higher level in advanced stage and aggressive tumors (28). Similarly, it is reported that high nm23 expression is correlated with the mortality rate and unfavorable clinical course (29). Other studies report that nm23 does not have prognostic value in ovarian carcinomas and is not associated with metastasis (30).

The nm23 expression rate was 67.9% in serous carcinomas, 64.3% in borderline serous tumors and 44.8% in benign serous tumors in our study. These rates are higher than in the literature. We found higher rates of nm23 expression in carcinomas and borderline tumors than benign tumors, although not statistically significant (p=0.116). When we evaluated the results in two separate groups as positive and negative, we found that 84.2% of the nm23 positive cases and 66.2% of the negative cases were at an advanced stage (p:0.032). This indicates that nm23 is expressed at a higher rate in advanced stage serous ovarian carcinoma cases in contrast to breast carcinoma and is acting like an oncogene. There are reports stating that nm23 is expressed in direct or inverse correlation with the malignancy potential. However, Schneider et al. has come up with a threshold pass theory (28) where it is stated that tumor malignancy is directly correlated with nm23 expression and that ovarian carcinomas are therefore in the same group as neuroblastomas and colon carcinomas (28). In addition to concurrence with the more malignant phenotype, nm23 also has an effect that decreases spread in these

tumors. nm23 is therefore expressed when the threshold value for malignancy is passed by the tumor and is one of the mechanisms that will prevent cellular spread. This explains why nm23 is an indicator of better prognosis in slow growing tumors such as breast carcinoma. However, ovarian carcinomas show rapid tumor progression and the threshold value is passed quickly. nm23 expression seems to appear in the late stage of ovarian carcinogenesis (28).

No significant relationship was found when grade and nm23 expression were compared in serous carcinomas but we found that tumors with a glandular growth pattern showed significantly less expression than those with papillary and solid growth (p=0.01) when tumor growth pattern, one of the grading system parameters, was compared with the nm23 expression. We can therefore say that there is less nm23 expression in more differentiated tumors. The nm23 expression was not different between groups classified according to their degree of pleomorphism or frequency of mitotic activity. These findings with serous tumors support literature data that nm23 is expressed at a higher level in tumors with an aggressive phenotype, that the role of nm23 in ovarian carcinomas is different than in breast carcinomas and that nm23 plays a tissue-specific role.

We found that serous carcinomas with p53 expression showed weak staining with nm23 which indicated an inverse relationship between p53 and nm23 but no statistical correlation could be demonstrated (r=0.067, p=0.579)

In conclusion, p53 plays a role in the carcinogenesis of serous tumors and appears at the late stage. The lack of a difference in bcl-2 expression in benign, borderline and malignant tumors and the inability to demonstrate a relationship between this expression and prognostic parameters such as stage and grade in carcinoma cases indicate that it does not play a role in ovarian tumor carcinogenesis. The higher nm23 expression in advanced stage and less differentiated (with solid and papillary growth pattern) ovarian tumors shows that it behaves like an oncogene in ovarian carcinomas.

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