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Original Article

Nm23 and HER2/neu gene expression and prognostic significance in urothelial carcinomas of the bladder

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Abstract

Aim: This study aimed to investigate the expressions of Nm23-H1 and HER2/neu in Turkish cases of urothelial carcinomas of bladder and to examine their associations with prognostic parameters. Urothelial carcinoma of the bladder is one of the most prevalent cancers worldwide. Nm23-H1 and HER2/neu expressions have been previously studied in urothelial carcinomas. Despite the availability of many reports in the literature, the prognostic significance of these expressions remains unclear.

Materials and Methods: Fifty cases of urothelial carcinoma of the bladder treated with radical cystectomy were retrospectively reviewed. The tumor specimens were evaluated by immunohistochemistry using Nm23-H1 and HER2/neu antibodies.

Results: Immunohistochemically, Nm23-H1 and HER2/neu expressions were present in 80% and 18% of cases and associated with pathological stage (p=0.025 and p=0.023, respectively).

Conclusion: Both antibodies were noted in patients with advanced stage cancer, which can adversely affect prognosis in infiltrative urothelial carcinomas of the bladder. HER2/neu may be a target for therapy.

Keywords: HER2/neu; Nm23-H1; pathologic stage; urothelial carcinoma

INTRODUCTION

Urothelial carcinomas of the bladder constitute the ninth most common cancer in the world according to the World Health Organization (WHO) data. Worldwide approximately 549.000 new cases and 200.000 deaths are detected every year (1). Etiological reasons are multifactorial, but the most common cause is exposure to smoking and aromatic amines. Smoking, particularly cigarette smoking, is a well-established risk factor for urothelial carcinoma of the urinary tract (2). It is more common in developed countries compared to developing countries, which is attributed to industrial and environmental pollution. More than 90% of bladder cancers are urothelial carcinomas originating from the surface epithelium of the bladder, with the rates of the other types of bladder cancer such as squamous cell carcinomas and adenocarcinomas being lower (3).

Several studies have attempted to identify the spectrum of genetic changes in urothelial carcinomas of bladder cancer and its associations with clinical outcome. Many important genes that cause bladder cancer and ensure its progression are yet to be discovered. Simultaneous overexpression and co-amplification of a large number of oncogenes are common. However, the clinical significance of these defects, either alone or in combination, is still not clear (4).

The Nm23-H1 gene is localized on chromosome 17q21.3. When it was first detected, it was considered as a metastasis suppressor gene (5). It was discovered in 1989 by Steeg et al., who is stated that it was less expressed in highly metastatic murine melanoma cells compared to non-metastatic cells (6). Later, Rosengard et al. identified the Nm23 gene with reduced RNA levels in tumor cells of high metastatic potential and referred to it as Nm23-H1 (7). Human epidermal growth factor receptor 2 (HER2/neu) is a transmembrane tyrosine kinase receptor involved in cell growth, survival and migration. A wide variability of HER2/neu overexpression has been reported to be generally related to a high grade and stage of bladder cancer and correlated with a poor prognosis (8,9). There are many studies discussing the prognostic significance of HER2/neu expression in malignancies, but there is still no consensus on its prognostic significance in some organ malignancies (9,10). There is a wide variability of HER2/neu overexpression in bladder cancer in studies in the literature (9). Our aim in this study was to examine the relationship between HER2/neu and Nm23-H1 expressions and prognostic parameters in bladder cancer and to contribute to the literature on this subject.

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MATERIALS and METHODS

We examined the samples of 50 patients who were diagnosed with urothelial carcinoma in the bladder between 2000 and 2006 in the Pathology Department of Atatürk University Faculty of Medicine and underwent cystectomy. Paraffin blocks, hematoxylin-eosin stained samples and pathological diagnosis reports of the cases were obtained from the archive of the department and re-evaluated according to the WHO/ISUP 2004 classification system in terms of depth of tumor invasion (pT), tumor grade, presence of lymphovascular invasion, macroscopic diameter, surgical margin assessment, lymph node assessment, and whether there were different areas of differentiation within the tumor. The most suitable paraffin blocks were selected for immunohistochemical staining.

NM23-H1 immunostaining (1/25 dilution DAKO Rabbit Anti-Human Nm23 protein A 0096, Denmark), CerbB2 (1/250 dilution, DAKO Rabbit Anti-Human CerbB2 Oncoprotein A, 0485, Denmark) was applied immunohistochemically to streptavidin-biotin peroxidase (Large volume DAKO LSAB 2 system-HRP kit, peroxidase DAKO in selected blocks (multiple blocks for some cases). For these applications, 4 micrometer-thick sections from paraffin blocks were placed on poly-L-lysine-coated slides. The sections were kept in the oven at 50 °C for one night. They were then deparaffinized in xyloles for 5 minutes, rehydrated in 70%, 80%, and 96% alcohols consecutively, and treated with 3% hydrogen peroxidase for 15 minutes to prevent endogenous peroxidase activity. The sections were washed with phosphate-buffered-saline (PBS) at a pH of 7.4. The tissues were immunostained with Nm23-H1 for 30 minutes and then washed with PBS again. As the secondary antibody, Biotinylated Link was treated for 15 minutes and washed with PBS. Streptavidin was treated with peroxidase for 15 minutes and washed with PBS, and then treated with DAB + chromogen for 6 minutes, washed with distilled water, stained with hematoxylin and washed again with distilled water, and sealed with an immunohistochemical closure solution. The same method was applied for HER2/neu (=CerbB2) immunostaining.

Evaluation of the immunohistochemical staining of Nm23-H1 and HER2/neu

Immunohistochemical staining preparations were first evaluated by two pathologists under a light microscope without using clinical and previous pathological data. The presence of cytoplasmic and/or nuclear yellowish staining for Nm23 -H1 was accepted as positive staining. All tumoral cells in tissue sections were evaluated for prevalence. The tissues were divided into four groups according to the amount of tumoral area, and 0-25% staining was evaluated as 0 (negative), 26-50% staining 1+, 51-75% 2+, and 76-100% 3+.

CerbB2 cytoplasmic membrane staining of more than 10% of tumor cells was considered positive. Membrane staining, partial and complete staining, as well as the severity of four separate groups were evaluated. The immunohistochemical staining characteristics for the HER-2/neu ratio of the resection materials were evaluated as follows: Score 0: no reactivity or membranous weak reactivity in <10% of tumoral cells, Score 1: weak or barely perceptible membranous reactivity in ≥10% of tumoral cells; Score 2: weak to moderately complete membranous reactivity in ≥10% membranous tumor cells; and Score 3: strong complete, persistent membranous reactivity in ≥10% of cancer cells.

Statistical Analysis

Statistical processes were carried out in computer environment using the Statistical Package for the Social Sciences software package. For this purpose, four groups were formed for Nm23-H1, four groups for CerbB2, four groups for pT, three groups for grade two groups for lymphovascular invasion, two groups for macroscopic diameter, and two groups for differentiation area. The results were evaluated with the Pearson-Spearman correlation test since there were not a sufficient number of cases in each group to obtain a more objective evaluation and the cases did not show a homogeneous distribution. If the probability coefficient (p) was equal to or less than 0.05, it was considered statistically significant.

RESULTS

Forty-five of the cases (90%) were male and five (10%) were female. The mean age was 60.0 ± 9.4 (range 37-78) years. The smallest tumor diameter was 2 cm, and the largest macroscopic diameter was 9 cm (mean 5.17 cm ± 1.62). Forty-five (90%) of the cases had a tumor diameter of >3 cm. Lymphovascular invasion was positive in 38 cases (76%). Four cases (8%) had lymph node metastases. When the cases were classified according to the WHO 2004 grading system, four (8%) were evaluated as grade 1, five (10%) as grade 2, and 41 cases (82%) as grade 3. Eight patients (16%) had positive surgical margins in the prostatic urethra, and one had positive surgical margins in the ureter.

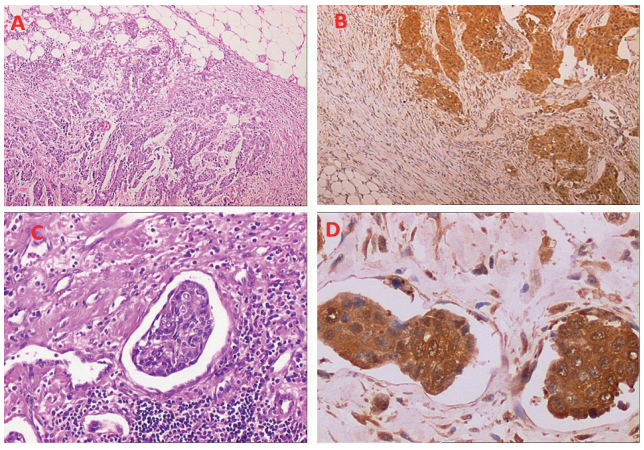

Figure 1. A- pT4 Perivesical adipose tissue invasion H&Ex40, B-Nm23-H1 positivity (3+) in the area of perivesical adipose tissue invasion Nm23-H1x100, C- Vascular invasion area H&Ex100, D-Nm23-H1 positivity in cells showing vascular invasion, Nm23-H1x100

Table 1. General characteristics and distribution of the cases

Case no	Sex	Age	Pathological stage (İnvasion dept-pT)	Vascular invasion	Lymph node status	Surgical margins	Tumor diameter (cm)	Nm23-H1 expressions	Nm23-H1 staining score	Differentiations	HER2/neu
1	M	78	4b	+	-	PU+	5	%90	+3	SD	Neg
2	M	60	2b	+	-	-	6	%60	+2	-	Neg
3	M	71	4b	+	-	-	9	%90	+3	SD+SarD	Neg
4	M	55	2a	+	-	PU+	6	%90	+3		Neg
5	M	48	3a	+	-	PU+	9	%80	+3	SD	Neg
6	M	55	3a	+	-	-	5	%40	+1	SD	Neg
7	M	58	3a	+	-	PU+	6	%90	+3	SD	1+
8	M	58	2a	+	-	-	4	%25	Neg		Neg
9	M	71	2a	-	-	-	3	%70	+2		Neg
10	M	37	2b	+	-	-	4	%70	+2		Neg
11	M	56	3a	+	-	-	6	%90	+3	SD	Neg
12	M	59	2b	+	-	-	5	%60	+2	SD	Neg
13	M	56	2b	+	-	-	3.5	%5	Neg		Neg
14	F	68	4b	+	-	-	6.5	%50	+1	Glandular	3+
15	M	71	2a	+	-	-	2	%20	Neg		Neg
16	M	50	2a	+	-	-	3.2	%40	+1		Neg
17	M	68	3a	+	4+	-	8	%20	Neg	SD	1+
18	M	71	4a	+	-	-	6	%80	+3	SD	2+
19	M	74	4a	+	-	-	4.5	%80	+3	SD	Neg
20	M	50	2a	-	-	-	4	%20	Neg	SD	Neg
21	M	52	2a	+	-	-	5.5	%80	+3		1+
22	M	65	4b	+	2+	-	5.5	%95	+3	SD	1+
23	M	58	4a	+	2+	-	7	%40	+1	SD	Neg
24	M	43	2a	-	-	-	4	%95	+3	SD	Neg
25	M	60	4a	+	7+	PU+	8	%95	+3	SD+SarD	Neg
26	M	68	3b	+	-	-	6	%90	+3	SD	Neg
27	M	60	2a	-	-	-	4	%0	Neg		Neg
28	F	65	1	-	-	-	4	%40	+1		Neg
29	M	57	3b	+	-	-	5	%10	Neg	SD	Neg
30	M	65	4a	+	-	-	4.5	%90	+3	SD	1+
31	M	77	3a	+	-	PU+	7	%10	Neg	SD	Neg
32	M	56	2a	-	-	-	6	%95	+3	SD	Neg
33	F	53	2b	+	-	-	6	%90	+3	SD	Neg
34	M	49	1	-	-	-	5	%20	Neg	SD	Neg
35	M	55	3a	+	-	-	2.7	%80	+3		Neg
36	M	75	4a	+	-	-	5.4	%90	+3	SD	Neg
37	M	71	2b	+	-	-	8	%40	+1		Neg
38	M	46	2a	-	-	-	4	%40	+1		Neg
39	M	69	3a	+	-	-	5	%95	+3	SD	3+
40	M	57	2b	+	-	-	4	%90	+3	SD	Neg
41	M	58	2a	-	-	-	2	%95	+3		Neg
42	M	58	3a	+	-	U+	6.2	%50	+1	SD	Neg
43	M	69	4a	+	-	-	6	%80	+3	SD	Neg
44	F	61	3a	+	-	-	5	%90	+3		Neg
45	M	66	2a	-	-	-	3	%90	+3	SD	Neg
46	M	61	3a	+	-	-	6	%20	Neg		1+
47	M	44	2b	-	-	-	4	%55	+2	SD	Neg
48	M	64	2b	-	-	PU+	3.5	%90	+3		Neg
49	F	58	4a	+	-	PU+	6	%70	+2	SD	Neg
50	M	46	2b	+	-	-	4.5	%60	+2	SD	Neg

PU: Prostatic urethra, SD: Squamous differentiation, SarD: Sarcomatoid differentiation, M: Male F: Female

Pathological stages were pT1b in two cases (4%), pT2a in 13 (26%), pT2b in 10 (20%), pT3a in 11 (22%), pT3b in two (4%), pT4a in eight (16%), and pT4b in four (8%). Squamous differentiation areas were present in 32 (64%), sarcomatoid differentiation areas in two (4%), and glandular differentiation areas in one (Table 1). There was a significant positive correlation between pathological stage and vascular invasion and tumor diameter ($p < 0.001$).

When Nm23-H1 expression was evaluated immunohistochemically according to the depth of invasion, 40 (80%) of 50 cases were observed to have different degrees of positivity. No staining was observed in one of the two pT1 cases, and only +1 staining was observed in the other. pT2a staining was detected in four (8%) cases 0, 1+ in two (4%), 2+ in one (2%), and 3+ in six (12%). Six of the 11 pT3a cases, one of the two pT3b cases, six of the eight pT4a cases, and all three pT4b cases had 3+ staining for Nm23-H1. As the pathological stage increased, Nm23-H1 staining increased (Figure 1 and Table 2). As the depth of invasion increased, the intensity and ratio of staining for Nm23-H1 were higher. There was a statistically significant relationship between pathological stage and Nm23-H1 expression ($p = 0.025$).

The immunohistochemical evaluation of nine of the 50 cases (18%) showed HER2/neu expression in different degrees. Similarly, there was a statistically significant relationship between pathological stage and HER2/neu expression ($p = 0.023$). Forty-one of the 50 cases (82%) HER2/neu was negative for HER2/neu. Most of the positive cases were pT3 and pT4. As in Nm23-H1 expression, with the increasing in pathological stage, the percentage and severity of HER2/neu staining also increased (Figure 2 and Table 3).

There was no significant relationship between Nm23-H1 and HER2/neu expressions and prognostic parameters, namely tumor diameter ($p=0.093$) lymphovascular invasion ($p=0.517$), lymph node metastasis and squamous ($p= 0.215$), and sarcomatoid differentiations ($p= 0.068$).

Table 2. Relationship between pathological stage (invasion depth) and Nm23-H1

	Pathological stage (invasion depth)							
Nm23-H1 expressions	**pT1**	**pT2a**	**pT2b**	**pT3a**	**pT3b**	**pT4a**	**pT4b**	**Total**
0 (negative)	1	4	1	3	1	-	-	**10**
1+	1	2	1	2	-	1	1	**8**
2+	-	1	5	-	-	1	-	**7**
3+	-	6	3	6	1	6	3	**25**
Total	2	13	10	11	2	8	4	**50**

Table 3. Relationship between pathological stage (invasion depth) and CerbB2

CerbB2 expressions	**pT1**	**pT2a**	**pT2b**	**pT3a**	**pT3b**	**pT4a**	**pT4b**	**Total**
0 (negative)	2	12	10	7	2	6	2	**41**
1+	-	1	-	3	-	1	1	**6**
2+	-	-	-	-	-	1	-	**1**
3+	-	-	-	1	-	-	1	**2**
Total	**2**	**13**	**10**	**11**	**2**	**8**	**4**	**50**

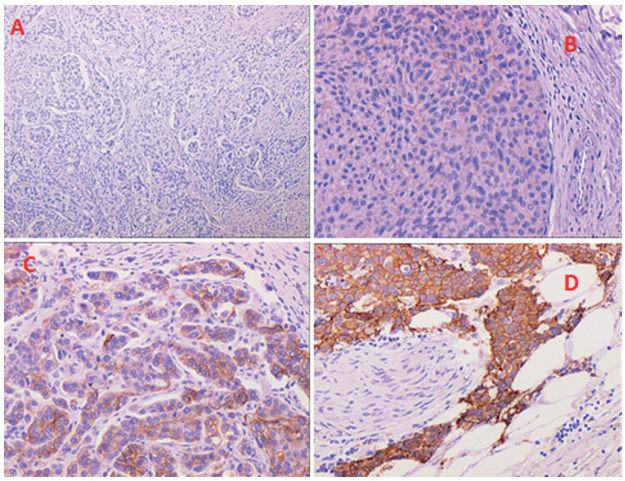

Figure 2. A- Negative HER2/neu x40, B- 1+ HER2/neu x100, C- 2+ HER2/neu x100, D- 3+ HER2/neu x100

DISCUSSION

Each year, around 549,000 new cases of bladder cancer are detected worldwide (1). The prevalence of bladder cancer increases with age, with the median patient age at diagnosis being 65-70 years, and it is also three to four times more common in males than in females (3,4). In our study, unlike previous work in the literature, the number of female patients was significantly lower, and the female/male ratio was 1/9. However, in accordance with the literature, the mean age of our cases was 60.0 ± 9.4 years.

The transformation of the normal urothelium into malignant cells and then metastasis is a complex process involving a very different number of genes, gene products, proteins and other molecules. There are many molecular methods and studies that examine the onset, progression and prognosis of bladder cancer (11,12). Detecting all

these genetic changes in advance will contribute to taking important preventive and therapeutic measures (13). It has been reported that aromatic amines (4-aminobiphenyl) found in cigarette smoke are the most known cause of bladder cancer and have a carcinogenic effect on the epithelium through DNA damage and mutation (14).

Eighty percent of bladder cancers are superficial (pTa, pTis, and pT1) while the remaining 20% (pT2 -pT4) are tumors that have invaded the muscle and have poor prognosis. The course of bladder cancers depends on the grade and stage of the initial tumor. However, the prognosis depends on the presence of tumor stage, grade, solid tumor morphology, multifocality, ureteric obstruction, positive pelvic lymph node, and tumor size being larger than 3 cm (11). The presence of mutations in tumor suppressor genes such as p53, p16, Rb and protooncogenes such as CerbB1, CerbB2, H-ras have been demonstrated in both superficial and muscle-invasive bladder tumors (11). Invasion depth forms the basis of the pT classification and is the most important prognostic factor in urothelial carcinomas. The more detailed classification of pT1 tumors is based on the level of invasion to the lamina propria. If the tumor goes beyond the lamina propria, it will progress rapidly and invade the muscle in a short time. High grade and stage tumors have a ten-year survival rate of 35% (4).

In agreement with the literature, of the 50 cases included in our study, 48 (96%) had muscularis propria and greater depth of invasion. Four (8%) of the cases were grade 1, five (10%) were grade 2, and 41 (82%) were grade 3. At the same time, all 38 cases with positive lymphovascular invasion were grade 3. Eight (16%) of these cases had tumor positivity in the prostatic urethra and one had surgical border positivity. Thirty-two (64%) of the cases with squamous differentiation, two cases with sarcomatoid differentiation and four cases with perineural-intraneural invasion were all grade 3. Furthermore, consistent with the literature, a significant relationship was found between the degree and depth of invasion and the presence of vascular invasion, macroscopic diameter, and squamous differentiation.

There are many genetic changes in invasive growing bladder urothelial carcinomas that cause damage in different areas of chromosomes. The most common chromosomal anomaly associated with bladder cancer is the deletions of Chromosome 9 (15). Many important genes that cause bladder cancer and ensure its progression are yet to be discovered. Simultaneous overexpression and coamplification of a large number of oncogenes are common. Among the oncogenes, HER2/neu, H-ras, cyclin-dependent kinases, and MDM2 are well known. H-ras mutations are observed in approximately 45% of bladder cancers depending on the detection method (16, 17). Tumor suppressor genes include p53, p63, PTEN, RB, E-cadherin, and Nm23-H1 (18, 19).

In the literature, there are some studies examining the relation of Nm23-H1 and CerbB2 expression with clinicopathological parameters and prognostic value in bladder cancers. However, the small number of such studies and the lack of research evaluating both molecules together warrant further investigations into these two molecules. In addition, the contradictory results reported in the literature regarding the Nm23-H1 gene make this gene expression more valuable in terms of examination. In our study, we found specific findings indicating the expression evaluation of Nm23-H1 and CerbB2 in areas of macroscopic diameter, lymphovascular invasion and squamous differentiation.

Nm23-H1 gene expression has been studied in many different human tumors; however, different results have been identified. For example, it has been suggested that the decrease or loss of Nm23-H1 expression in tumors such as breast, prostate, cervix, colon and stomach cancers will increase the potential of metastasis and have poor prognostic value (19-22). It has been reported that Nm23-H1 is highly expressed in all hematological malignancies, which is associated with a poor prognosis. It has also been emphasized that high Nm23-H1 expression, especially AML-M5 has prognostic significance (23).

In our study, as mentioned in the results section, of the 23 pT2 cases, nine (40%) had 3+, seven (30%) had 2+, two (9%) had 1 +, and five (21%) had 0 (negative) Nm23-H1 expression. Among the 13 pT3 cases, 3+ Nm23-H1 expression was present in seven (54%), 2+ in one (8%), 1+ in two (15%) and 0 (negative) in three (23%). For the pT4 group, of the 12 cases, nine (75%) had 3+, one (8.3%) had 2+ and two (16.6%) had 1+ Nm23-H1 expression. These data indicate that all pT4 tumors had Nm23-H1 expression. In addition, a correlation was observed between the depth of invasion and the high expression of Nm23-H1.

The literature contains conflicting data on the expression of Nm23-H1 in bladder carcinomas. While some studies show that Nm23-H1 expression increases as the depth of invasion increases, others suggest that there is higher expression in superficial tumors. Although we determined that Nm23-H1 expression increased as the depth of invasion increased in our study, the effectiveness of our findings are limited by our sample consisting of only patients with cystectomy and the low number of cases with superficial tumors. Therefore, it is necessary to conduct further studies with more homogeneously distributed cases.

Decreased Nm23-H1 expression in esophageal squamous cell carcinomas was previously reported to be associated with a short survival time. In the same study, Nm23-H1 expression was shown to be low or negative in blood vessel invasion (24). In a study comparing the Nm23-H1 expression of benign and malignant ovarian tumors, it was observed that the Nm23-H1 expression of malignant and metastatic tumors decreased significantly compared to the benign group. Similarly, malignant and metastasized cases had lower Nm23-H1 expression than those without metastasis (25).

In a study including 185 colorectal carcinomas, Dursun et al. found weak Nm23-H1 positivity in 31 (17%) cases,

moderate in 48 (26%), and strong in 106 (57%). The authors stated that well-differentiated adenocarcinomas showed stronger positivity than moderately or poorly differentiated adenocarcinomas (26). It was also reported that with the increase in tumor stage, Nm23-H1 expression decreased and Nm23-H1 expression was weaker in cases with lymphovascular invasion, lymph node and liver metastasis. The disease-free and overall life span of the cases with low Nm23-H1 expression were determined to be shorter than those with strong Nm23-H1 expression, and based on these findings, the authors concluded that the degree of Nm23-H1 expression could be a useful prognostic parameter (26). In contrast, we observed high Nm23-H1 expression in areas of vascular invasion.

Kanayama et al. evaluated 22 bladder cancerous tissue and seven cell culture samples and found that the expression of Nm23-H1 and H2 was higher in both tumor human tissues and cell cultures compared to normal bladder mucosa. In addition, higher Nm23-H1 expression was observed in grade 2 bladder tumors than grade 1 tumors (27). Our results were consistent with these findings. In another study by Shiina et al., it was investigated whether PCNA, p53, nuclear DNA amount and Nm23-H1 expression could constitute prognostic data in 77 (pTa-pT3b) urothelial carcinoma cases. In this review, it was stated that Nm23-H1 had no prognostic significance (5).

Chow et al., evaluating 257 bladder cancer cases, stated that there was a significant difference between pTa tumors and pT1-3 tumors, and observed decreased Nm23-H1 expression as the depth of invasion increased, which was associated with a decreased lifetime and increased risk of metastasis (28). These results are not compatible with the data of our study.

In an immunohistochemical study, among 45 pTa-T1 and 42 pT2-T4 bladder transitional cell carcinomas, Nakopoulou et al. showed higher rates of Nm23-H1 positivity in the tissue samples of invasive and high-grade cases, and these patients were also reported to have a worse prognosis (29). These findings are in agreement with our results in terms of grade and invasivity.

In 39 bladder carcinoma cases treated with cystectomy, Kuo et al. found higher Nm23-H1 positivity in tumors with a higher stage but similar to our study, they reported no relation between patient age and sex and tumor morphology (30).

HER2/neu overexpression is observed in 30-70% of patients with invasive bladder cancer. Although some researchers claim that HER2/neu expression is an indicator of metastasis development or lifespan, these findings have not been confirmed by other investigators (31). In our study, nine (18%) cases with positive staining with HER2/neu were muscle invasive cases. However, despite the relationship between muscle invasion and HER-2/neu, there was no significant relationship between grade and HER-2/neu expression. Jimenez et al. examined HER2/neu expression in primary and metastatic urothelial carcinoma of the bladder among 80 cases. HER-2/neu was found positive in 28% of primary tumors and 58% of metastatic tumors. The mean survival time was reported to be 33 months in HER2/neu-positive tumors and 50 months in HER-2/neu-negative tumors (32). In our study, higher (3+) positivity was observed in muscle invasive tumors, consistent with the previous research.

Gorgolius et al. suggested that the simultaneous high expression of CerbB2 and EGF was associated with an increased histological grade and stage of the disease and aggressive behavior (33), which is similar to our results. In a study by Kruger et al., HER-2/neu gene amplification and overexpression were examined in 203 muscle-invasive urothelial carcinoma cases, and overexpression was detected in 37% of the cases and gene amplification in 5%. High HER-2/neu expression was found to be significantly associated with tumor grade, infiltrative growth pattern and lymph node metastasis. Disease-free survival was reduced in the presence of high HER-2/neu expression. The authors suggested that HER-2/neu was an important tool in immunohistochemically determining the prognosis of bladder carcinomas (34). These results are consistent with our research findings on lymph node metastasis and invasivity.

Myomato et al. detected HER2/neu gene amplification in 18 (32%) of 57 bladder cancers using the polymerase chain reaction test. It was reported that cases with gene amplification had higher grade and stage and a shorter survival time. In light of this information, the authors concluded that when the evaluation of HER-2/neu amplification and tumor stage and grade together would create independent prognostic data since tumors with high grade and HER-2/neu amplification showed more aggressive behavior (35).

Coogan et al. examined HER2/neu gene amplification and overexpression in transitional cell carcinoma in 54 (pTa-T3) bladder cancer cases and reported the expression rate as 48% in grade 3 tumors and 14% in grade 1 tumors. While 17% overexpression was observed in pTa- pT1 tumors, high expression was detected at a rate of 44% in pT2-3 tumors. Based on these findings, the authors reported that high HER-2/neu expression might be an important prognostic finding (36), which is compatible with our results concerning tumor grade and invasivity.

It is generally stated in the literature that HER2/neu expression increases in invasive (pT2-4) bladder cancers, and this may constitute a prognostic parameter and treatment target. In our study, we obtained similar results to the literature.

CONCLUSION

Our study involved a unique evaluation of the expression of an oncogene (HER-2/neu) and an antioncogene (Nm23-H1) in urothelial carcinomas of the bladder. There was no statistically significant relationship between Nm23-H1 and HER-2/neu expression. However, it is remarkable that the expression of both molecules increased with the depth of invasion, which is the most important prognostic

factor. On the other hand, the association between the expression of both molecules with grade and superficial lesions remains controversial. We consider that this is due our cases being selected only from those that underwent cystectomy and had infiltrative lesions. Nevertheless, the high expression of both Nm23-H1 and HER-2/neu is important in invasive (pT2-pT4) cases and may have prognostic and therapeutic value for this group. Therefore, it is necessary to conduct further studies involving more non-invasive urothelial carcinoma cases.

Competing Interests: The authors declare that they have no competing interest.

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Ethical Approval: This research was ethically approved by Atatürk University Faculty of Medicine ethic committee with decision number B.30.2.ATA.0.01.00/63-09.06.06.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Zeegers MP, Kellen E, Buntinx F, et al. The association between smoking, beverage consumption, diet and bladder cancer: a systematic literature review. World J Urol 2004;21:392-401.
3. Kirkali Z, Chan T, Manoharan M, et al. Bladder cancer: epidemiology, staging and grading, and diagnosis. Urology 2005;66:4-34.
4. Moch H, Cubilla AL, Humphrey PA, et al. The 2016 WHO classification of tumours of the urinary system and male genital organs—part A: renal, penile, and testicular tumours. Eur Urol 2016;70:93-105.
5. Shiina H, Igawa M, Nagami H, et al. Immunohistochemical analysis of proliferating cell nuclear antigen, p53 protein and nm23 protein, and nuclear DNA content in transitional cell carcinoma of the bladder. Cancer 1996;78:1762-74.
6. Steeg PS, Bevilacqua G, Kopper L, et al. Evidence for a novel gene associated with low tumor metastatic potential. JNCI 1988;80:200-4.
7. Rosengard AM, Krutzsch HC, Shearn A, et al. Reduced Nm23/Awd protein in tumour metastasis and aberrant Drosophila development. Nature 1989;342:177-80.
8. Hansel DE, Swain E, Dreicer R, et al. HER2 overexpression and amplification in urothelial carcinoma of the bladder is associated with MYC coamplification in a subset of cases. Am J Clin Pathol 2008;130:274-81.
9. El Ochi MR, Oukabli M, Bouaiti E, et al. Expression of human epidermal growth factor receptor 2 in bladder urothelial carcinoma. BMC clinical pathology 2017;17:3.
10. Ceylan O , Ozmen S . Prognostic Significance of HER-2 Expression in Gastric Cancer. Black Sea Journal of Health Science 2021;4:52-57
11. Kumar V, Abbas AK, Fausto N, et al. Robbins and Cotran pathologic basis of disease, professional edition e-book: Elsevier health sciences 2014.
12. Ceylan O, Karabulut I. Importance of cytokeratin-20 expression in papillary urothelial neoplasia. Cukurova Med J 2020; 45:1326-1332
13. Mhawech-Fauceglia P, Cheney RT, Schwaller J. Genetic alterations in urothelial bladder carcinoma: an updated review. Cancer 2006;106:1205-16.
14. Kadlubar FF, Badawi AF. Genetic susceptibility and carcinogen-DNA adduct formation in human urinary bladder carcinogenesis. Toxicol Lett 1995;82:627-32.
15. Lapham RL, Grignon D, Ro JY, editors. Pathologic prognostic parameters in bladder urothelial biopsy, transurethral resection, and cystectomy specimens. Semin Diagn Pathol 1997.
16. Lipponen P, Eskelinen M. Expression of epidermal growth factor receptor in bladder cancer as related to established prognostic factors, oncoprotein (c-erb B-2, p53) expression and long-term prognosis. Br J Cancer 1994;69:1120-5.
17. Bos JL. Ras oncogenes in human cancer: a review. Cancer Res 1989;49:4682-9.
18. Khaled HM, Bahnassy AA, Raafat AA, et al. Clinical significance of altered nm23-H1, EGFR, RB and p53 expression in bilharzial bladder cancer. BMC cancer 2009;9:32.
19. Simon R, Struckmann K, Schraml P, et al. Amplification pattern of 12q13-q15 genes (MDM2, CDK4, GLI) in urinary bladder cancer. Oncogene 2002;21:2476-83.
20. Leone A, Flatow U, King CR, et al. Reduced tumor incidence, metastatic potential, and cytokine responsiveness of nm3-transfected melanoma cells. Cell 1991;65:25-35.
21. Bertheau P, De La Rosa A, Steeg PS, et al. NM23 protein in neoplastic and nonneoplastic thyroid tissues. Am J Pathol 1994;145:26.
22. Lacombe M-L, Sastre-Garau X, Lascu I, et al. Overexpression of nucleoside diphosphate kinase (Nm23) in solid tumours. Eur J Cancer Clin Oncol 1991;27:1302-7.
23. Yokoyama A, Okabe-Kado J, Wakimoto N, et al. Evaluation by multivariate analysis of the differentiation inhibitory factor nm23 as a prognostic factor in acute myelogenous leukemia and application to other hematologic malignancies. Blood, Comparative Study 1998;91:1845-51.
24. Tomita M, Ayabe T, Matsuzaki Y, et al. Expression of nm23-H1 gene product in esophageal squamous cell carcinoma and its association with vessel invasion and survival. BMC cancer 2001;1:1-6.
25. Kapitanovic S, Spaventi R, Vujsic S, et al. nm23-H1 gene expression in ovarian tumors--a potential tumor marker. Anticancer Res 1995;15:587-90.
26. Dursun A, Akyurek N, Gunel N, et al. Prognostic implication of nm23-H1 expression in colorectal carcinomas. Pathology 2002;34:427-32.

27. Kanayama Ho, Takigazva H, Kagazva S. Analysis of nm23 gene expressions in human bladder and renal cancers. Int J Urol 1994;1:324-31.
28. Chow N-H, Liu H-S, Chan S-H. The role of nm23-H1 in the progression of transitional cell bladder cancer. Clin Cancer Res 2000;6:3595-9.
29. Nakopoulou L, Constandinides C, Tzonou A, et al. Dimopoulos C. Immunohistochemical evaluation of nm23-H1 gene product in transitional cell carcinoma of the bladder. Histopathology 1996;28:429-35.
30. Kuo J, Chiang H, Chen K, et al. Immunohistochemical analysis of nm23-H1 protein in bladder cancer. Zhonghua yi xue za zhi. Chin Med J 1999;62:411-7.
31. Lönn U, Lönn S, Friberg S, et al. Prognostic value of amplification of c-erb-B2 in bladder carcinoma. Clin Cancer Res 1995;1:1189-94.
32. Jimenez RE, Hussain M, Bianco FJ, et al. Her-2/neu overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic significance and comparative analysis in primary and metastatic tumors. Clin Cancer Res 2001;7:2440-7.
33. Gorgoulis VG, Barbatis C, Poulias I, et al. Molecular and immunohistochemical evaluation of epidermal growth factor receptor and c-erb-B-2 gene product in transitional cell carcinomas of the urinary bladder: a study in Greek patients. Mod Pathol 1995;8:758-64.
34. Krüger S, Weitsch G, Büttner H, et al. Overexpression of CerbB2 oncoprotein in muscle-invasive bladder carcinoma: relationship with gene amplification, clinicopathological parameters and prognostic outcome. Int J Oncol 2002;21:981-7.
35. Miyamoto H, Kubota Y, Noguchi S, et al. CERBB2 gene amplification as a prognostic marker in human bladder cancer. Urology 2000;55:679-83.
36. Coogan CL, Estrada CR, Kapur S, et al. HER-2/neu protein overexpression and gene amplification in human transitional cell carcinoma of the bladder. Urology 2004;63:786-90.