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Correlation of Human Epididymis Protein 4 Expression with Clinicopathologic Parameters in Gastric Carcinomas

Mide Karsinomlarında İnsan Epididim Proteini 4 Ekspresyonunun Klinikopatolojik Parametrelerle İlişkisi

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ABSTRACT

Background: Human epididymis protein 4 (HE4) was discovered in 1991 as a glycoprotein secreted from the cells of the human epididymal epithelium and associated with sperm development and immunity. Subsequent studies have shown that HE4 is also expressed in many normal tissues such as reproductive, respiratory, and digestive tract epithelia. Although the mechanism of action of HE4 in cancers is still unknown, its increased expression has been reported in many tumors, especially gynecologic malignancies. In our study, we have investigated the relationship between gastric carcinomas and increased HE4 expression.

Materials and Methods: HE4 expression was studied in 114 formalin-fixed paraffin-embedded gastric carcinoma specimens and its association with different clinicopathologic parameters was evaluated.

Results: Immunohistochemical HE4 expression was strong in 88 patients and weak in 26 of 114 patients. A significant correlation was found between HE4 staining intensities and five-year survival rates (p=0.002). There was no significant correlation between HE4 staining intensity and human epidermal growth factor 2 (HER2)/neu amplification, as well as other clinicopathologic data.

Conclusion: This study has demonstrated the association of HE4 expression with 5-year survival in gastric tumors. In addition, although no significant correlation was found between HE4 staining intensity and HER2/neu amplification in our study, a significant correlation between these parameters has been reported in the literature. In conclusion, HE4, which we have found to be associated with long-term survival in our study, can be used as a prognostic marker in gastric cancers.

Keywords: Gastric cancer, HE4, HER2/neu

ÖZ

Amaç: İnsan epididim proteini 4 (HE4), 1991 yılında insan epididim hücrelerinden salgılanan sperm gelişimi ve immüniteyle ilişkili bir glikopotein olarak keşfedilmiştir. Daha sonraki çalışmalarda HE4, reprodüktif, solunum ve sindirim yollarının epiteli gibi birçok normal dokularda da eksprese edildiği görülmüştür. HE4'ün kanserlerdeki mekanizması halen kesin olarak bilinmemesine rağmen, başta jinekolojik maligniteler olmak üzere birçok tümörde ekspresyonun artığı bildirilmiştir. Araştırmamızda mide karsinomları ile HE4 ekpresyon artışı arasındaki ilişkiyi inceledik.

Gereç ve Yöntemler: Formalinle fikse edilmiş parafine gömülü 114 mide karsinomu örneğinde HE4 ekspresyonu çalışıldı ve farklı klinikopatolojik parametrelerle ilişkisi değerlendirildi.

Bulgular: İmmünohistokimyasal HE4 ekspresyonu 114 olgumuzun 88'inde güçlü, 26'sında zayıf olarak izlendi. Beş yıllık sağkalım süresi ile HE4 boyanma şiddetleri arasında anlamlı ilişki bulundu (p=0,002). HE4 boyanma şiddeti ile insan epidermal büyüme faktörü 2 (HER2)/neu amplifikasyon ve diğer klinikopatolojik veriler arasında anlamlı ilişki saptanmadı.

Sonuç: Bu çalışma mide tümörlerinde HE4 ekspresyonunun 5 yıllık sağkalım ile ilişkisini göstermiştir. Ayrıca çalışmamızda HE4 boyanma şiddeti ile HER2/neu amplifikasyon arasında anlamlı ilişki bulunmamış olsa da literatürde anlamlı ilişki bulunduğu

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ÖZ

bildirilmektedir. Sonuç olarak, çalışmamızda uzun süreli sağkalımla ilişkili bulduğumuz HE4, mide kanserlerinde prognostik bir belirteç olarak kullanılabilir.

Anahtar Kelimeler: Mide kanseri, HE4, HER2/neu

Introduction

Gastric cancer is the fourth most common cancer in the world and is still the second leading cause of cancer-related mortality despite the decline in its incidence in recent years (1). Gastric cancer is a multifactorial disease. Environmental factors contributing to carcinogenesis include *Helicobacter pylori* (*H. pylori*) infection, low socioeconomic status, and the consumption of smoked and low-fiber foods (1-3). Genetic processes play more prominent roles in diffuse-type adenocarcinomas (1-3). Gastric cancer is 2-3 times more common in men than in women. The incidence of gastric cancer also varies between geographical regions. In countries with a low incidence of gastric adenocarcinomas, diffuse type and proximal location are more common, while in countries with a high incidence, intestinal type and distal location are more common (1-3). Mostly, acquired genetic alterations are involved in gastric carcinogenesis. Mutations in the K-ras oncogene and *adenomatous polyposis coli* gene are seen in adenoma, intestinal metaplasia, and intestinal-type gastric cancer, but not in diffuse gastric cancer. Instead, allelic loss of the *TP53* gene is seen in 60% of cases, and it is the most common genetic alteration detected in gastric cancer (4). Amplification of c-erbB-2, a transmembrane tyrosine kinase receptor oncogene, encoded by the *human epidermal growth factor 2* (*HER2*) gene, is reported in 10-30% of mainly intestinal type gastric carcinomas in different series and indicates poor prognosis (5-7). The incidence of gastric cancer increases with age and is extremely rare before the age of 30. These young patients are mainly females with diffuse-type cancer. Intestinal-type adenocarcinomas, on the other hand, are common in older male patients. While the five-year survival rate was 15% in the past years, this rate has reached 30% today (1,6,7).

Human epididymis secretory protein 4 (HE4) was first identified in 1991 as a glycoprotein secreted by human epididymal epithelial cells and associated with sperm development and immunity. In subsequent studies, HE4 has been demonstrated in a wide variety of epithelial cells including reproductive epithelium, respiratory epithelium, salivary gland mucus cells, mammary ductal epithelium, and lung epithelium (8). It is expressed at a lower rate in kidney, prostate, pituitary, and thyroid cells, particularly in the distal tubule of kidney. HE4 belongs to the whey acidic proteins (WAP) family. WAP-like proteins belong to a small group of heterogeneous, acidic, heat-stable proteins with diverse biological functions. The basis of their biological function lies in their binding capacity to membrane receptors. A significant number of them also show protease-inhibitory activity (8,9). HE4 expression has been shown to be increased in gynecologic malignancies, lung, and breast cancers (10-12).

HE4 was approved as a serum tumor marker in ovarian carcinomas by the US Food and Drug Administration in 2003. Although increased HE4 expression has been shown in many malignancies, the role of this protein in gastric cancer is not yet clearly known (13,14).

The objective of this study is to investigate the correlations between HE4 expression and different clinicopathologic variables of gastric carcinomas, with a focus on HER2 expression status.

Materials and Methods

A total of 114 gastrectomized gastric carcinoma patients, diagnosed in the Pathology Laboratory of University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital between 2011 and 2014, with sufficient archival material were included in the study. Informed consent was obtained for this study. This study was ethically approved by the Local Ethics Committee of the University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital (approval number: 1-11, date: 26.01.2017). Age, gender, tumor location, tumor diameter, TNM stage, and overall survival data were obtained from pathology records. Hematoxylin-eosin (H&E) stained slides of all cases were re-evaluated according to the 2019 World Health Organization classification system (6). The staging system most often used for stomach cancer is the American Joint Committee on Cancer (AJCC) TNM system, which was last updated in 2018 (7). Clinical prognostic factors such as tumor type, grade, stage, lymphovascular, and perineural invasion, and lymph node involvement were evaluated. In addition, c-erbB2 immunohistochemical (IHC) expressions and HER2 amplification have been re-assessed according to the American Society of Clinical Oncology/College of American Pathologists 2013 guidelines on the archived slides.

Among the paraffin blocks of the cases, the one that best reflected the tumor tissue for IHC staining was selected.

The area to be analyzed was marked first on the slide and then on the block. Tissue samples with a diameter of 2 mm were taken from the labeled areas on donor paraffin blocks and transferred to microarray blocks by a manual mapping-addressing technique using a microarray device. H&E sections were first taken from the prepared multiple blocks, and the presence of tumors in the sampled areas was confirmed. Then, 4-micrometer-thick sections were taken onto polylysine-coated slides and manually stained with the anti-HE4 antibody. The sections were kept in an oven at 60 °C overnight. Antigen retrieval was performed by heating prepared slides in citrate buffer (pH: 6.0) in a microwave oven at 400 Watt for 20 minutes in plastic chalices with closed lids. Primary antibody anti-HE4 (1:20 dilution; Signet Laboratories Inc., Dedham, MA, USA) was applied for 1 hour. Manual staining was performed using the streptavidin-biotin method. All cases demonstrated cytoplasmic staining for HE4. Therefore, a quantitative evaluation could not be made. HE4 cytoplasmic staining intensities were considered weak or strong.

Statistical Analysis

The SPSS 22.0 program (IBM Corporation, Armonk, NY, USA) was used to statistically analyze the variables. Quantitative variables are shown as mean ± standard deviation and categorical variables as percentages (%). A Student's t-test was used in independent two-group comparisons in which quantitative data were evaluated. Pearson chi-square, linear-by-linear association, and Fisher's exact test were used in the comparisons of categorical variables in which qualitative data were evaluated. The results of analyses were considered statistically significant at $p<0.05$ within a 95% confidence interval. The Kaplan-Meier methods and the log-rank (Mantel-Cox) test were used to test the effects of HE4 staining status on survival.

Results

Demographic and histopathologic data were obtained by evaluating the information available in the pathology laboratory records of 114 patients, including 36 (31.6%) female and 78 (68.4%) male cases. The mean age at diagnosis was 63.6±12.3 years (range: 36-89 years). During a mean follow-up period of 29.7±20.6 (0-89) months, 37 (32.5%) patients survived, and 77 (67.5%) patients died. Tumors were located in the corpus (n=57; 50%), pylorus (n=36; 31.6%), and cardia (n=21; 18.4%). The mean tumor diameter was 6.2±2.9 cm (1-15 cm). In our study, diffuse type adenocarcinoma (n=46; 31.6%), intestinal type adenocarcinoma (n=71; 62.3%), and mixed type adenocarcinoma (n=7; 6.1%) were detected in the indicated number of cases. The tumors diagnosed were poorly (n=60; 52.7%) and moderately differentiated (n=54; 47.3%). Only 5 cases (4.9%) had neuroendocrine differentiation. Tumor necrosis was observed in 6 (5.3%), lymphovascular invasion in 74 (64.9%), and perineural invasion in 69 (60.5%) cases. Lymph node metastasis was present in 90 (78.9%) cases. The pT distribution of our cases according to the 2018 AJCC TNM staging system is as follows: pT1b (n=7; 6.1%), pT2 (n=6; 5.3%), pT3 (n=68; 59.6%), and pT4 (n=33; 28.9%). Our patients were in the early (n=13; 11.4%) and advanced (n=101; 88.6%) stages of the disease. Distant organ metastases were present in 33 (28.9%) cases. Most frequently, liver (n=15; 13.2%) and then pulmonary (n=11; 9.6%), peritoneal (n=5; 4.4%), and ovarian (n=2; 1.8%) metastases were seen.

Immunohistochemically, c-erbB2 expression status was investigated in all 114 cases. HER2/neu amplification was also evaluated at a molecular level by FISH test in cases. Immunocytochemically, 2+ or 3+ positive c-erbB2 expression was detected in 28 cases (24.6%). However combined evaluation of both immune histochemical c-erbB2 expression and HER2 amplification, only 21 cases (18.4%) were evaluated as positive. The HE4 staining results were evaluated in terms of staining intensity in indicated percentages of cells as follows: 0, negative: 0-10%; 1+, weak: 11-30%; 2+, moderate: 31-60%, and 3+, strong: 61-100%. However, since all 114 cases, showed 61-100% staining intensities, a proportional evaluation could not be made. Due to the small number of cases and the insufficient number of cases in the groups, HE4 cytoplasmic staining intensities 0 and 1+ were considered as weak while 2+ and 3+ staining intensities as strong staining and all parameters were compared according to cytoplasmic staining intensity. According to this evaluation, all cases demonstrated cytoplasmic staining. Among 114 patients, 26 (22.8%) cases demonstrated weak and 88 (77.24%) cases strong staining intensities for HE4 (Figures 1-3).

We investigated the relationship between IHC HE4 staining intensities and clinicopathologic parameters of diagnostic, therapeutic, and prognostic importance, in 114 patients who underwent gastrectomy for gastric cancer patients (Table 1). HE4 staining intensities were not associated with gender (p=0.919), mean age at diagnosis (p=0.420), localization (p=0.916), diameter (p=0.551), histological type of tumors (p=0.498), and degree of tumor differentiation (p=0.402), no statistically significant correlation was found between the presence of neuroendocrine differentiation (p=0.680), tumor necrosis, lymphovascular invasion (p=0.682), perineural invasion (p=0.136), and lymph node metastasis (p=0.167). There was no statistically significant correlation between tumor stage and HE4 staining intensity according to the 2018 AJCC TNM staging system (p=0.686). Also, no statistically significant

correlation was detected between distant organ metastasis (p=0.816), the organ of metastasis (p=0.586), HE4 staining intensity. No statistically significant correlation was found between IHC c-erbB2 staining, which is used to evaluate HER2/neu amplification, and HE4 staining intensity (p=0.848). Any statistically significant difference was not observed in terms of HE4 staining intensity between the group of patients with positive, and negative HER2/neu amplification detected by FISH test (p=0.179). No statistically significant correlation was noted between survival times and HE4 staining intensities (p=0.190). However, the mean 5-year survival times of the patient groups demonstrating weak and strong staining intensities were 54.7 and 64.03 months, respectively. Accordingly, a statistically significant correlation was detected between survival times and HE4 staining intensities (p=0.028) (Figure 4).

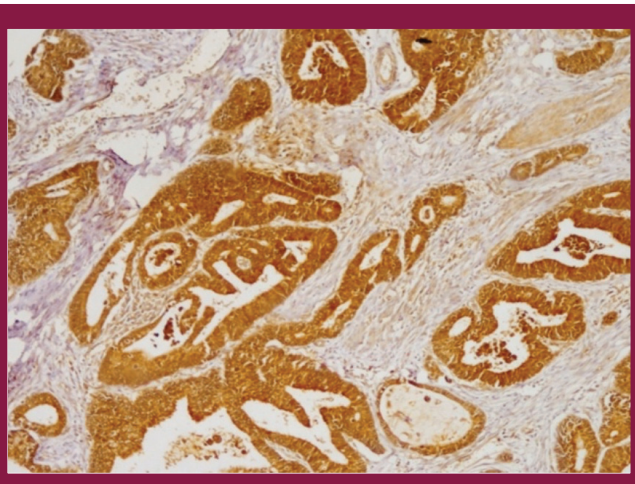

Figure 1. Immunohistochemically detected strong cytoplasmic HE4 staining in a case with intestinal type adenocarcinoma (DAB, x200)
HE4: Human epididymis protein 4

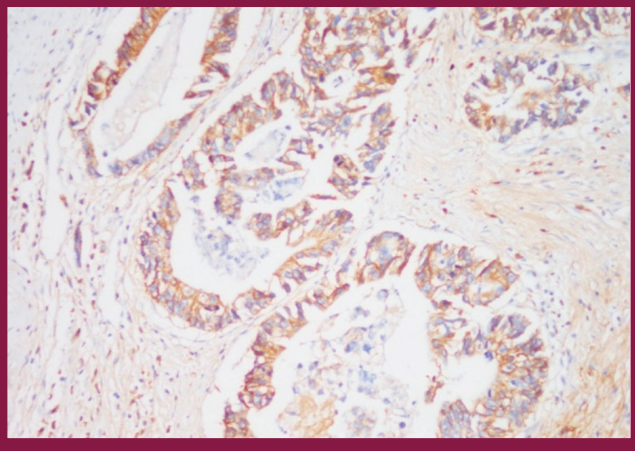

Figure 2. Immunohistochemically detected weak cytoplasmic HE4 staining in a case with intestinal-type adenocarcinoma (DAB, x200)
HE4: Human epididymis protein 4

Discussion

Diet, genetics, *H. pylori* infection, chronic gastritis, gastric dysplasia, intestinal metaplasia, and surgical damage are known risk factors involved in the complex etiology

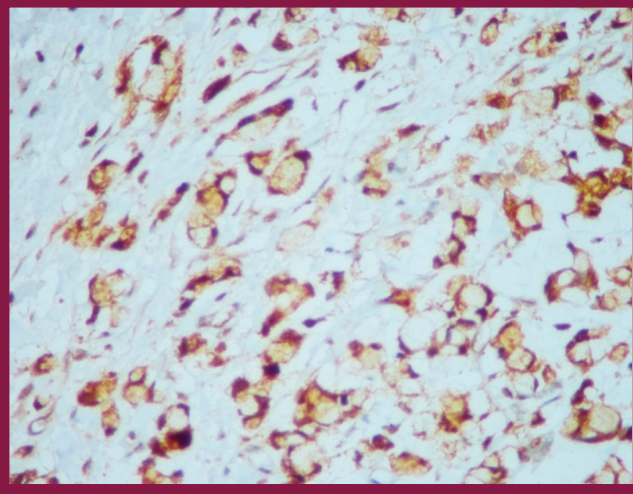

Figure 3. Immunohistochemically detected strong cytoplasmic HE4 staining in a case with diffuse-type adenocarcinoma (DAB, x200)
HE4: Human epididymis protein 4

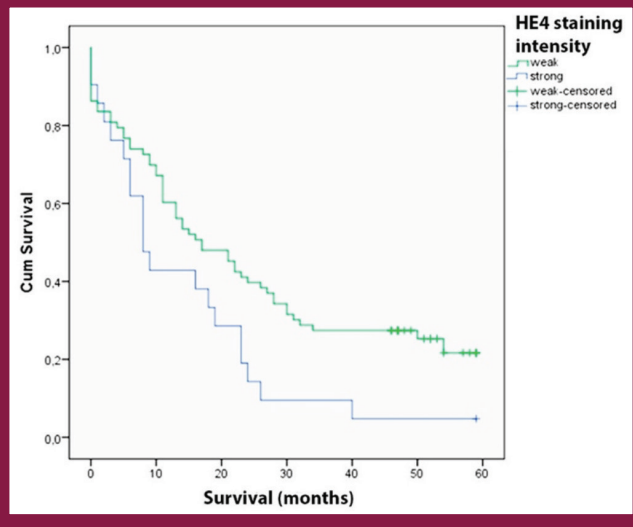

Figure 4. Differences in 5-year survival rates according to HE4 staining intensities (log-rank test, p=0.028)
HE4: Human epididymis protein 4

and pathogenesis of gastric cancers, but the significance of these parameters has not been fully elucidated (1-4). Gastric cancer is one of the most common cancers with fatal outcomes in the world. The survival rate is quite low, as 90% of the cases are in the advanced stage at the time of diagnosis. Due to the lack of simple and sensitive markers for early diagnosis, the optimal treatment window is often overlooked (1).

Recent studies have focused on identifying new molecular markers both to predict survival and recurrence in gastric cancer and to develop relevant active treatment methods (13,14). HE4 is a marker of proven prognostic importance in many malignancies, especially gynecologic cancers (15-20). There are few studies in the literature on the prognostic importance of HE4 in gastric carcinoma (13,14). In our study, the statistically significant prolonged survival time in patients with poor HE4 expression suggests that HE4 expression is a marker that may have prognostic significance in gastric cancer.

Many of the biological functions of proteins encoded by the *HE4* gene are linked to their capacity to bind to membrane receptors. A significant number of them also exhibit protease inhibitor activity. IHC studies have shown that this protein is located in the cytoplasm of cells (8,9). In addition, *HE4* gene expression has been shown to be increased in tumors compared to normal ovarian tissue, particularly in ovarian tumors (12). Although the precise functional mechanism of HE4 involved in cancers is still unknown, its increased expression and secretion have been described in a wide variety of epithelial tumors including epithelial ovarian carcinomas, bronchial adenocarcinomas, and breast tumors. Apart from these carcinomas, increased

Table 1. Findings of patients according to the HE4 expression

Severity of HE4 expression		Strong; n, % (88: 77.2%)	Weak; n, % (26: 22.8%)	p-value
Gender	Male	60/68.2	18/69.2	0.919
	Female	28/31.8	8/30.8	
Tumor location	Cardia	16/18.2	5/19.2	0.633
	Corpus	46/52.3	11/42.3	
	Pylor	26/29.5	10/38.5	
Survival status	Deceased	57/64.8	20/76.9	0.245
	Survived	31/35.2	6/23.1	
Lymph node metastases	Absent	16/18.2	8/30.8	0.167
	Present	72/81.8	18/69.2	
Location of distant metastases	Absent	63/71.6	18/69.2	0.816
	Liver	12/13.6	3/11.5	
	Lung	9/10.2	2/7.7	
	Periton	3/3.4	2/7.7	
	Ovary	1/1.1	1/3.8	
Tumor stage	Early	11/12.5	2/7.7	0.391
	Late	77/87.5	26/92.3	
Histology	Intestinal-type	53/60.2	18/69.2	0.498
	Poorly adhesive/mixed	35/39.8	8/30.8	
Lymphovascular invasion	Present	58/65.9	16/61.5	0.682
Perinueral invasion	Present	50/56.8	19/73.1	0.136
HER2 simple (according to ASCO/CAP 2013 criteria)	Negative	71/80.7	22/84.6	0.179
	Positive	17/19.3	4/15.4	
Age (year)	Mean ± SD	62.3±12.1	67.8±12.4	0.420
Tumor diameter (cm)	Mean ± SD	6.2±3	5.9±2.9	0.551
Survival (months)	Mean ± SD	26.6±20.8	22.7±20.3	0.190
Patients with longer survival	5 years and over	54.6±11.5	64±4.7	**0.028**

HE4: Human epididymis protein 4, HER2: Human epidermal growth factor 2, ASCO/CAP: American Society of Clinical Oncology/College of American Pathologists, SD: Standard deviation

expression of the *HE4* gene has also been reported in mesothelioma, gastrointestinal tumors, and melanoma (21-24). HE4 is also used as a biomarker in the differentiation between benign and malignant ovarian neoplasms and the determination of their types. Indeed, its expression is increased in epithelial ovarian cancers, but not in non-epithelial ovarian tumors. Currently, HE4 is used as a tumor biomarker in epithelial ovarian carcinomas in combination with cancer antigen-125 (25). High serum HE4 levels are associated with the development of ascites, chemoresistance, and decreased survival in ovarian malignancies. It is known that HE4 elevation in ascites fluid, as well as in serum, has significant implications (26). Similarly, in our study, gastric carcinoma patients with strong HE4 expression in tumor cells had shorter survival times.

There is increasing evidence that HE4 may be an effective biomarker not only in ovarian carcinomas but also in other gynecologic cancers. For example, although HE4 expression is present in normal endometrial tissues, HE4 protein levels are also increased in endometrial cancer. Expression levels of HE4 in endometrial carcinoma are associated with poor prognosis. Significantly higher HE4 expression levels have been detected in atypical hyperplasia, which is one of the precursor lesions of the endometrial tumor, compared with the healthy control group. Accordingly, it has been claimed that serum HE4 concentration in endometrial cancer patients may give an idea about the diameter of the primary tumor and depth of myometrial invasion (18). Studies examining the association of HE4 with neoplasms of the cervix have found increased HE4 expression levels in normal cervical epithelium and invasive tumors, in contrast to lower expression levels in intraepithelial carcinoma (19). All these studies suggest that HE4 is an implicated gene in the development of gynecologic cancers (18-20).

The relationship between breast cancer and HE4 has been investigated in recent years. A study published in 2016 showed that serum HE4 levels were higher in breast cancer patients compared with healthy individuals (23,27). Akoz et al. (17) found a significant correlation between cytoplasmic HE4 staining intensity and c-erbB2 staining status, HER2/neu amplification, as well as an inverse relationship with tumor grade. HER2/neu amplification is an indicator of poor prognosis in gastric cancer. The presence or absence of amplification changes the treatment protocol. Therefore, evaluation of HER2/neu amplification, which is an indicator of poor prognosis and leads to the creation of new treatment targets, is recommended in all gastric cancer cases. No case reports on the relationship between HER2/neu amplification and HE4 expression have been cited in the literature so far. Similarly, we couldn't find any statistically significant relationship between HE4 staining intensity and the presence or absence of HER2/neu amplification using both c-erbB2 expression by immunohistochemistry and HER2 amplifications by in situ hybridization. Studies suggest that HE4, whose biological function has not yet been clarified, is a gene involved in the process of carcinogenesis. HE4 expression has been effectively used in the diagnosis and therapy of gynecological cancers. Especially, in cancers of the digestive system, HE4 expression has been overlooked in the English literature. Therefore, the current articles have mentioned the HE4 expression, in terms of differential diagnosis (12). A limited number of studies have investigated HE4 expression in gastric cancer (13,14). O'Neal et al. (13) reported that normal gastric mucosa could not be immunohistochemically stained for HE4, but HE4 expression was detected in epithelium with intestinal metaplasia which indicates a step toward malignant progression. When gastric cancer patients were compared with the healthy group, it was found that both the intensity and percentage of IHC HE4 staining increased in cancer patients. According to Lauren's classification of gastric tumors, diffuse-type gastric cancer is stained more strongly than intestinal-type gastric cancer. Diffuse-type cancers have a worse prognosis than intestinal-type cancers. In light of this information, it has been suggested that HE4 may be an indicator of poor prognosis (13). In our study, no significant correlation was found between Lauren's classification of gastric tumors and increased HE4 expression. Since 77.8% of diffuse-type cancers stained strongly for HE4, further studies with larger case series would likely confirm these findings to be consistent with the literature. O'Neal et al. (13) found a negative correlation between increased HE4 expression, and survival in the second group consisting of patients in Western countries. Similarly, increased HE4 expression was inversely correlated with 5-year survival in our study.

A study by Guo et al. (14) in 2014 evaluated the relationship between increased HE4 expression and clinicopathologic parameters of gastric cancer, as presented in the literature. In this study, increased HE4 expression was observed in gastric cancer patients in accordance with the literature. Among the prognostic markers, age, gender, stage of the disease, lymph node metastasis, and tumor invasion (pT) were not found to be significantly associated with HE4 staining intensities (14). In our study, no correlation was found between the above-mentioned parameters and HE4 staining intensities. However, they found that overall survival time decreased as HE4 staining intensity increased, and strong HE4 staining and overall survival time were inversely correlated with each other. In our study, no correlation was found among Lauren's classification of tumors, tumor size, and increased HE4 expression. In the survival analysis of our study, 70%

of our patients were not alive when the statistical analyses were performed, and the median follow-up period was 29.2 months. The survival analysis of the patients according to HE4 staining intensities could not reveal any significant difference between the median survival times. However, we examined the relationship between 5-year survival times and HE4 staining intensities and found that the 5-year survival times of strongly stained cases were significantly decreased compared to weakly stained cases, consistent with the literature findings. As the HE4 staining intensities or HE4 expression levels increased, 5-year survival times decreased significantly.

HE4 expression has been more effectively used in the diagnosis and therapy of gynecological cancers. Especially in the cancer of the digestive system, HE4 expression was seen to be overlooked in the English literature. Therefore, current articles have mentioned the HE4 expression in terms of differential diagnosis.

Study Limitations

The most important limitation of this study is that HE4 immunostaining was applied only in microarray blocks prepared from small tumor samples. For this reason, staining intensity could not be compared with the surrounding tissue, and no comment could be made as to whether the expression detected in the tumor was also present in the normal mucosa.

Conclusion

Although the functional mechanism involved has not been fully elucidated, all these studies have shown that the *HE4* gene is effective in the processes of carcinogenesis. The absence of HE4 expression in healthy gastric epithelial cells and its increased expression during the progression of gastric cancer, suggest that the use of this parameter may shed light on early diagnosis and treatment protocols in gastric cancer. The inverse correlation between HE4 expression and 5-year survival in our study suggests that HE4 may be a marker for the diagnosis, prognosis, and treatment of gastric cancer patients. Therefore, studies on HE4 expression in gastric cancer should be conducted in large series.

Ethics

Ethics Committee Approval: This study was ethically approved by the Local Ethics Committee of the University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital (approval number: 1-11, dated: 26.01.2017).

Informed Consent: Informed consent was obtained for this study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: P.Ö., G.D., T.A., Y.K., D.A., Concept: P.Ö., G.D., D.A., Design: P.Ö., G.D., D.A., Data Collection or Processing: P.Ö., G.D., T.A., Y.K., D.A., Analysis or Interpretation: P.Ö., G.D., Literature Search: P.Ö., G.D., T.A., Y.K., D.A., Writing: P.Ö., G.D., T.A., Y.K., D.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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