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Evaluation of Immunohistochemical HIF-1α Expression in Gastric Adenocarcinomas According to Clinicopathological Parameters

Mide Adenokarsinomlarında İmmünohistokimyasal HIF-1α Ekspresyonunun Klinikopatolojik Parametrelerle İlişkisinin Değerlendirilmesi

Talya Akata[1], Gülden Diniz[2], Pınar Öksüz[3], Yetkin Koca[4], Samir Abdullazade[5]

[1]University of Health Sciences Turkey, İzmir Suat Seren Chest Diseases Hospital, Department of Pathology, İzmir, Turkey
[2]İzmir Democracy University, Buca Seyfi Demirsoy Research Hospital, Department of Pathology, İzmir, Turkey
[3]Ankara Bilkent City Hospital, Clinic of Pathology, Ankara, Turkey
[4]Malatya Forensic Medicine Institution, Department of Pathology, Malatya, Turkey
[5]Christian-Albrechts-University, University Hospital Schleswig-Holstein, Department of Pathology, Kiel, Germany

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Abstract

Objective: The alpha subunit of hypoxia-inducible factor-1 (HIF-1α) activates glucose transport, glycolytic enzymes, and transcription of genes encoding vascular endothelial growth factor (VEGF). HIF-1α expression is a mechanism by which tumor cells adapt to hypoxia. Gastric cancer is one of the most common cancers worldwide and has the highest mortality rate. Because patients are usually in their advanced stages at the time of diagnosis, the survival rates are low. The present study aimed to evaluate the prognostic significance of tissue expression of HIF-1α in gastric adenocarcinomas.

Methods: In this study, immunohistochemical HIF-1α expression was analyzed in gastrectomy materials from 114 gastric adenocarcinomas.

Results: HIF-1α expression was detected in 24 cases (21.1%). One (p=0.02) and five-year (p=0.03) survival rates were higher in cases with HIF-1α expression. In the regression analysis, the risk of death was 3.42 times higher in patients with advanced pathologic tumor stages (pT3 and pT4). Age, sex, tumor size, tumor location, lymphovascular and perineural invasion, HER2 expression, and other clinicopathological parameters were not significantly correlated with HIF-1α expression and survival.

Conclusion: It is important to identify specific prognostic markers and new targeted treatment options for gastric cancer. Despite conflicting findings, HIF-1α expression is recognized as a negative prognostic factor in many malignancies, and therapeutic agents that may be effective, especially in the HIF-1α/VEGF pathway, have been developed. However, the survival rate of patients with HIF-1 expression was higher in patients with HIF-1α expression. A greater amount of data should be retrieved from further research studies on the prognostic significance of HIF-1α expression in gastric carcinomas, especially after the standardization of immunohistochemical evaluation methods of its expression.

Keywords: Stomach, gastric carcinoma, HIF-1α, HER2

Address for Correspondence/Yazışma Adresi: Assoc. Prof. Gülden Diniz, İzmir Democracy University, Buca Seyfi Demirsoy Research Hospital, Department of Pathology, İzmir, Turkey
E-mail: gulden.diniz@idu.edu.tr
ORCID ID: orcid.org/0000-0003-1512-7584

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Öz

Amaç: Hipoksi ile indüklenen faktör-alfa alt ünitesinin (HIF-1α) aktivasyonuyla glukoz transportunu, glikolitik enzimleri ve vasküler endotelyal büyüme faktörü (VEGF) kodlayan genlerin transkripsiyonunu aktive eder. HIF-1α ekspresyonunun tümör hücrelerinin hipoksiye adaptasyon mekanizmalarından biri olduğu gösterilmiştir. Mide kanserleri dünyada en sık görülen ve mortalitesi en yüksek kanserler arasında yer almaktadır. Tanı anında genellikle ileri evrede olduğu için sağkalım oranı oldukça düşüktür. Bu çalışmada mide adenokarsinomlarında HIF-1α doku ekspresyonunun prognostik öneminin değerlendirilmesi amaçlanmıştır.

Yöntem: Bu çalışmada, 114 mide adenokarsinom olgusunun gastrektomi materyallerinde immünohistokimyasal HIF-1α ekspresyonu incelenmiştir.

Bulgular: Olguların 24'ünde (%21,1) HIF-1α ekspresyonu saptanmıştır. HIF-1α ekspresyonu saptanan olgularda bir (p=0,02) ve beş yıllık (p=0,03) sağkalım daha yüksek bulunmuştur. Regresyon analizinde patolojik tümör evresi ileri tümörlerde de (pT3 ve pT4) ölüm riski 3,42 kat daha yüksek bulunmuştur. Yaş, cinsiyet, tümör boyutu, tümör yerleşimi, lenfovasküler ve perinöral invazyon, HER2 ekspresyonu ve diğer klinikopatolojik parametreler ile HIF-1α ekspresyonu ve sağkalım açısından istatistiksel olarak anlamlı sonuç bulunmamıştır.

Sonuç: Mide kanserlerinde spesifik prognostik belirleyicilerin ve yeni hedefe yönelik tedavi seçeneklerinin belirlenmesi çok önemlidir. Çelişkili bulgular olsa da; HIF-1α ekspresyonu pek çok malignitede olumsuz bir prognostik faktör olarak değer görmektedir ve özellikle HIF-1α/VEGF yolağında etkili olabilecek tedavi ajanları geliştirilmiştir. Oysa çalışmamızda HIF-1α ekspresyonu saptanan olgularda sağkalım oranı daha yüksek bulunmuştur. Gastrik karsinomlarda HIF-1α ekspresyonunun prognostik önemiyle ilgili, özellikle immünohistokimyasal değerlendirmenin standardizasyonu sonrası yapılacak daha çok çalışma ve veriye ihtiyaç vardır.

Anahtar Kelimeler: Mide, gastrik karsinom, HIF-1α, HER2

Introduction

Gastric cancer is the fifth most frequently occurring type of cancer worldwide. Despite its decreasing incidence, it still ranks fourth in terms of cancer-related mortality[1]. Gastric cancer is a multifactorial disease and is primarily caused by *Helicobacter pylori (H. pylori)* infection, diet, alcohol consumption, smoking, Ebstein-Barr virus infection, and genetic factors are effective in its development[2,3]. Human epidermal growth factor receptor 2 (HER2), also known as "erythroblastosis oncogene B2 (ERBB2)", is a proto-oncogene encoded by the *ERBB2* gene located on chromosome 17[4]. HER2 overexpression is a common molecular abnormality in gastric cancers. Although its prognostic significance for gastric cancers is debatable, the detection of its overexpression in tumors has gained importance with the development of targeted therapies[5].

Tumor hypoxia plays a key role in the progression of malignancy, and hypoxia-inducible factor-1 (HIF-1) acts as a master regulatory molecule in the adaptation of cells to changing levels of oxygen. HIF-1 is composed of α and β subunits of the basic helix-loop-helix transcription factor family. The β subunit is synthesized as a basic component, and its activity is controlled by an oxygen-independent mechanism. In contrast, the α subunit (HIF-1α) is ubiquitinated and degraded under normoxic conditions and stabilized under hypoxia. Under hypoxic conditions, HIF-1α activates many hypoxia-responsive elements, especially vascular endothelial growth factor, and stimulates pathways necessary for tumor progression[6,7].

The aim of this study was to determine the possible prognostic significance of HIF-1α in gastric cancer, to investigate its relationship with clinicopathologic parameters, and to contribute to the identification of HIF-1α as a therapeutic target molecule.

Materials and Methods

A total of 114 cases of gastric adenocarcinoma diagnosed in the medical pathology laboratory of a research hospital between 2011 and 2014 were included in the study. Data related to age, sex, tumor location, tumor diameter, presence of lymph node metastasis, and TNM stage were obtained from pathology records. The presence of distant metastasis and survival data were retrieved from the hospital's electronic patient file system. Hematoxylin&eosin stained slide preparations of all cases were re-examined for tumor type, grade, pathological stage, and lymphovascular and perineural invasion (PNI). Immunohistochemically stained slides to assess HER2 expression were re-evaluated. Patients whose clinical and follow-up data were not available and whose tumor tissue material was insufficient for analysis were excluded from the study. The study was approved by the Local Ethics Committee of the University of Health Sciences Turkey, İzmir Tepecik Education and Training Hospital (approval number: 2017/14-37, dated: 11.12.2017). Informed consent was obtained from all patients prior to surgery to allow the use of the surgical materials obtained for scientific purposes.

Paraffin block, which is most suitable for IHC evaluation and best reflects the characteristics of tumor tissue, was selected

for the analysis. Next, paraffinized cylindrical tissue samples with a diameter of 2 mm were taken from the donor blocks by marking them first on the slide and then on the block. Multiple blocks (microarray blocks) were prepared using mapping and addressing techniques. From the prepared blocks, 4-m thick sections were placed on latinized slides. One section was stained with hematoxylin and eosin and the other was manually stained with polyclonal HIF-1α primary antibody (ATLAS, 1/300 dilution, catalog number: HPA001275). After deparaffinisation in an oven at 60 °C for 12 h, the sections were boiled in citrate solution for 20 minutes at 65 °C in a PT LINK device. Slides were allowed to cool in buffer solution for 5 min. Sections incubated with antibody for 1 h were manually stained using biotin-avidin peroxidase method (Invitrogen, Camarillo, CA, USA). Inflammatory cells in the sections also showed positive nuclear staining and were used as a positive internal control. HIF-1α expression was not observed in every tumor or field of view. Nuclear or nucleocytoplasmic staining was considered as evidence of HIF-1α expression[8]. Since HIF-1α expression was heterogeneously distributed, no quantitative grading was performed, and expression was only evaluated as "present" or "absent" (Figure 1).

Statistical Analysis

Statistical analysis was performed using the IBM SPSS 22.0 statistical package program. P≤0.05 was accepted as the level of significance. For the comparison of quantitative data, the chi-square test was used. In the comparison of quantitative parametric data independent groups, the t-test and non-parametric data Mann-Whitney U test were used. The non-parametric Kruskal-Wallis test was utilized for the comparison of more than 2 groups. For survival analysis, survival probabilities were tested with the Kaplan-Meier method, and the log-rank test was performed to determine whether there was a difference between survival probabilities. Cox regression analysis was then performed to identify factors affecting survival.

Results

The study population included 72 (63.2%) male and 42 (36.8%) female patients aged between 36 and 92 years, with a mean age at diagnosis of 63.8 years. Gastric adenocarcinomas were located in the cardia in 20 (17.5%), corpus in 54 (47.4%), and antrum/pylorus in 40 (35.1%) cases. The mean tumor size was 6.33 ±3.2 cm (range: 1-15 cm). According to the World Health Organization 2019 classification, the distribution of histological subtypes of tumors was as follows: poorly cohesive carcinoma (n=36; 31.6%), tubular (n=52; 45.6%), papillary (n=5; 4.4%), mucinous (n=12; 10.5%), and mixed (n=9; 7.9%) gastric adenocarcinomas. According to Lauren classification, the distribution of the histological types of the tumors was as follows: Diffuse type (n=36; 31.5%), intestinal type (n=69; 60.5%), and indeterminate type (n=9; 7%). According to pathologic TNM classification, adenocarcinomas were in stages pT4 (n=35; 30.7%), pT3 (n=66 cases; 57.9%), pT2 (n=6; 5.3%), and pT1b (n=7; 6.1%). Lymphovascular (n=74; 64.9%), perineural (n=68; 59.6%), and local lymph node metastasis

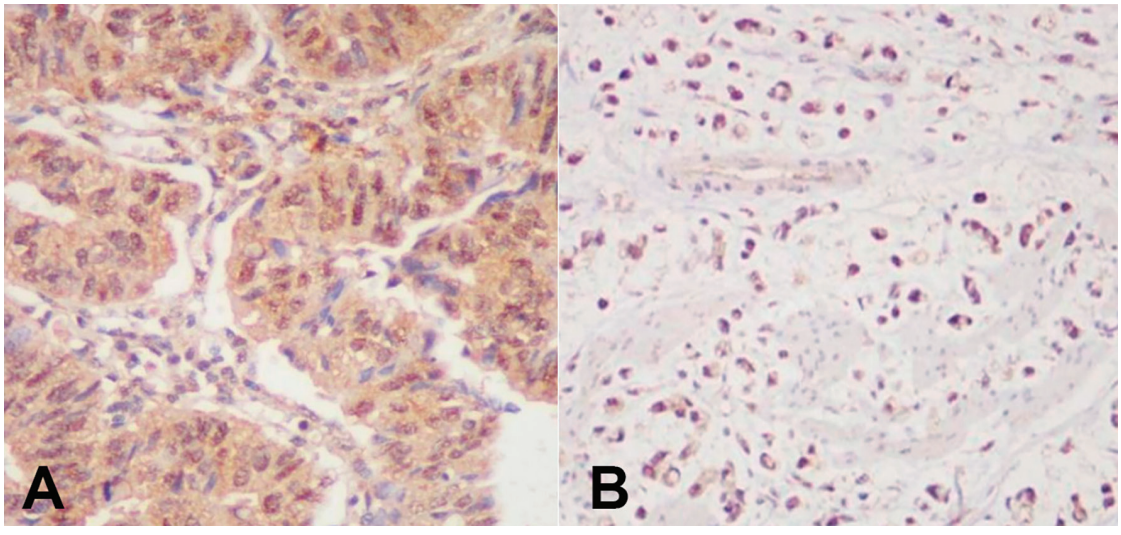

Figure 1. A) HIF-1α expression in tubular adenocarcinoma and B) in a diffuse (poorly cohesive) carcinoma specimen (DAB,200x)

HIF-1: Hypoxia-inducible factor-1

(n=89; 78.1%) were detected in the indicated number of cases. The number of metastatic lymph nodes ranged from 1 to 44 (mean 7.5±8.8, median 7 lymph nodes). The study population consisted of patients with stage N0 (n=25; 21.9%), N1 (n=14; 12.3%), N2 (n=29; 25.4%), N3a (n=24; 21.1%), and N3b (n=22; 19.3%) gastric adenocarcinoma. Distant organ metastases were observed in 35 (30.7%) patients, and they were localized in the lungs (n=11), liver (n=15), peritoneum (n=7), and ovaries (n=2). Immunohistochemical HIF-1α expression was detected in 24 of 114 patients (21.1%). The relationship between clinicopathological findings and HIF1a expression in patients is presented in Table 1.

Table 1. Association between qualitative and quantitative findings of patients according to HIF-1A expression status

HIF1-A expression		**Absent M/SD**	**Present M/SD**	**p**
Age (year)		64.1±12.7	63.1±9.8	0.740
Tumor diameter (cm)		6.6±3.3	5.3±2.75	0.830
Number of metastatic lymph nodes		9.5±8.4	9.4±9.3	0.380
Survival (months)		23.6±22.1	36.5±24.3	0.101
HIF1-A expression		**Absent N/%**	**Present N/%**	
Gender	**Male** **Female**	55/61.1 35/38.9	17/70.8 7/29.1	0.380
Tumor type	**Intestinal** **Diffuse** **Others**	52/57.7 32/35.6 6/6.7	17/70.8 4/16.8 3/12.4	0.170
Tumor location	**Cardia** **Corpus** **Antrum/pylori**	15/16.6 42/46.6 33/36.6	5/20.9 12/50 7/29.1	0.760
HER2 positivity (with IHC)	**Negative (- or +)** **Score 2 (++)** **Score 3 (+++)**	81/90 6/6.6 3/3.3	21/87.6 1/4.1 2/8.3	0.710
Extensive necrosis	**Absent** **Present**	88/97.8 2/2.2	21/87.6 3/12.4	0.062
Lymphovascular invasion	**Absent** **Present**	34/37.7 56/62.3	6/25 18/75	0.240
Perineural invasion	**Absent** **Present**	35/38.9 55/61.1	11/45.8 13/54.2	0.530
Lymph node metastases	**Absent** **Present**	18/20 72/80	7/29.1 17/70.8	0.330
Distant metastases	**Absent** **Present**	59/65.5 31/34.4	20/83.2 4/16.8	0.130
Location of distant metastases	**Liver** **Lung** **Periton** **Ovary**	10/11.1 14/15.5 5/5.5 2/2.2	1/4.1 1/4.1 2/8.3 -	0.918
Tumor stage	**Early** **Late**	8/8.9 82/91.1	5/20.9 19/79.1	0.100
Survival status	**Deceased** **Survived**	67/74.4 23/25.6	13/54.2 11/45.8	0.054

HIF-1: Hypoxia-inducible factor-1, SD: Standard deviation, IHC: HER2 immunohistochemistry, HER2: Human epidermal growth factor receptor 2

HER2 immunohistochemistry (IHC) scores were 0 or 1+ in 102 (89.5%) cases, and both groups were considered HER2-negative. IHC scores of 2+ and 3+ were detected in 6.1% (n=7) and 4.4% (n=5) of the cases, respectively. While HIF-1α expression was observed in 3 (25%) HER2- positive, but in 21 (20.58%) HER2- negative cases. There was no statistically significant correlation between HER2- negative and positive groups and HIF-1α expression (p=0.71). Similarly, there were no statistically significant differences in HIF-1α expression according to most clinicopathological features, such as sex (p=0.38), age (p=0.74), tumor location (p=0.76), histological type of tumors (p=0.17), presence of lymph node metastasis (p=0.33), pathological tumor stage (p=0.10), tumor size (p=0.83), lymphovascular invasion (LVI) (p=0.24), PNI (p=0.53), presence of extensive tumor necrosis (p=0.062), and presence of distant metastases (p=0.13). Although there was no statistical relationship between the presence of hif1 expression and the mean survival time and survival status, the difference was significant when compared with the median survival time. The reason for this can be explained by the fact that the survival times of the patients were in a very wide range, and the standard deviation was large. On the other hand, the median survival times were 41.4 and 15.4 months in HIF-1α positive, and negative cases, respectively, and a statistically significant (p=0.03) difference was detected between both groups in terms of HIF-1α staining status. Contrary to most studies in the literature, the survival time was longer in our patients with HIF-1α-expressing tumors (Figure 2). In terms of HIF-1α staining status, the 1- and 5-year survival rates in HIF-1α positive, and negative groups were 45.8% vs. 36.9% and 25.6% vs. 22.3%, respectively, with statistically significant intergroup differences (p=0.02 vs. 0.03).

Discussion

The incidence of gastric cancer significantly increases with age. Most patients are diagnosed between the ages of 60 and 80 years. Gastric cancers are rarely diagnosed in patients aged 45 years and are defined as "early-onset gastric cancer". It is believed that early-onset cases have different clinicopathological characteristics and develop in different models of carcinogenesis[9]. The mean age at diagnosis in our study population was 63.89 years, which is consistent with the literature findings. Only 7% of our patients were under 45 years of age at the time of diagnosis.

Gastric cancer is twice as common in men as in women. In addition, they are the fourth and fifth most common causes of cancer-related deaths in men and women, respectively[1-3]. The gender distribution of the study population was consistent with the literature findings. The process of gastric carcinogenesis is multifactorial, and both environmental and genetic factors play a role in the development of gastric cancers[9]. Approximately 89% of all gastric tumors are associated with helicobacter pylori infection[2].

Although gastric tumors seem to represent a single disease state, they can be considered as two entities "cardia" and "non-cardia" gastric cancers based especially on their widely different etiopathogenesis. Although HP infection, low socio-economic status, and dietary factors are blamed for the development of non-cardia gastric cancers, obesity and gastroesophageal reflux disease are believed to play a role in the etiology of gastric cardia cancers. Age, male sex, family history, smoking, and radiation exposure are common risk factors for tumors located in both regions[2,5,10-12]. Gastric cancer localization frequently varies according to the geographic region in which the patient lives. Although distally localized tumors i.e., in the corpus, antrum, and pylorus) constitute the majority of cases in the geographic regions where gastric cancer is endemic, tumors localized in the cardia and fundus are slightly more common in other geographic regions[12]. It is known that the prognosis of gastric cardia cancer is worse than that of distal gastric tumors because they are usually diagnosed at an advanced stage[9].

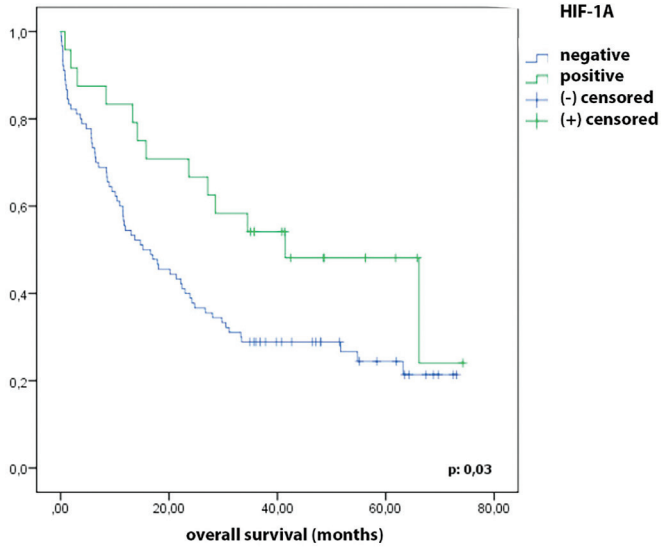

Figure 2. The relationship between HIF-1α expression and overall survival (Log rank, p=0.03)

HIF-1: Hypoxia-inducible factor-1

Although 20 (17.5%) cases of gastric carcinomas included in our study were located in the cardia/fundus, 94 (82.5%) were located in the distal stomach. A statistically significant difference was not detected in survival times between patients with gastric tumors of different localizations.More than 90% of gastric cancers have adenocarcinoma morphology, and the most common histologic type is tubular adenocarcinoma according to the World Health Organization classification. When the Lauren classification is taken into consideration, intestinal-type gastric carcinoma was more common in the past, whereas in recent years, especially in some countries, the incidence rates of diffuse and intestinal carcinoma seem to be close to each other[2]. Similarly, 45.6% of our cases had tubular adenocarcinoma morphology, followed by poorly cohesive carcinomas with a frequency of 31.6%. According to Lauren classification, most of the cases were intestinal type carcinomas (60.5%).

TNM staging alone is the most important prognostic factor of gastric cancer. Although 5-year survival is >90% in patients with pT1 tumors without lymph node metastasis, this rate decreases to 30% in those with pT3 tumors[2]. Our patients had pT1 and pT2 (n=13; 11.4%), pT3 (n=66; 57.9%), and pT4 (n=35; 30.7%) adenocarcinomas. When pT1 and pT2 cases were included in the early-stage gastric adenocarcinoma group and pT3 and pT4 cases in the late-stage gastric adenocarcinoma group, the median survival was 55 months in patients with pT1 and pT2 tumors, whereas it was 27.9 months in those with pT3 and pT4 tumors, with a statistically significant difference between groups (p=0.006).

The lymph node status is not only important in determining the stage of gastric cancer but also in indicating the need for adjuvant treatment. Recent studies have shown that "metastatic lymph node ratio", defined as the ratio of metastatic lymph nodes to total resected lymph nodes, may be an independent prognostic factor for gastric cancers[13]. Our study population of 114 patients were included in stages N0 (n=25; 21.9%), N1 (n=14; 12.3%), N2 (n=29; 25.4%), N3a (n=26; 21.1%), and N3b (n=22; 19.3%) based on the number of metastatic lymph nodes. Although the median survival time of patients without lymph node metastasis was significantly longer, the intergroup difference was not statistically significant.

Despite the fact that LVI is one of the most important factors in the development of lymph node metastasis in gastric carcinomas, data on its effect on survival as an independent factor remain controversial[14]. LVI was observed in 52 (31.9%) of 114 patients. In terms of survival, the median survival time was slightly longer in patients without LVI (20.1 months) than in those with LVI (18 months). However, the difference between the two groups was not statistically significant. Although PNI can predict recurrence in gastric cancers, there is not enough data on its prognostic value. Deng et al.[15] reported that PNI can be considered an independent prognostic factor in a meta-analysis of 24 studies including 30,590 patients who underwent curative gastrectomy. In the present study, PNI was found in 43 patients (25.9%). The median survival time was 17.9 months in patients with PNI and 24.7 months in those without. The difference between the two groups was not statistically significant in terms of median survival time.

Approximately 40% of gastric cancers are metastatic at the time of diagnosis, and the median survival for untreated metastatic gastric cancers is 4 months, whereas the 5-year survival is 3-6%[16]. Metastases commonly spread to the lungs and liver via hematogenous routes. Distant metastasis was found in 35 (30.7%) patients. In parallel with the literature, these metastases were most commonly observed in the liver, lungs, peritoneum, and ovaries. The median survival times were 24.2 months in patients without distant metastases and 14.7 months in patients without. Although this intergroup difference was remarkable and consistent with the literature, it was not statistically significant.

One of the most important molecules for targeted gastric cancer treatment is HER2. HER2 participates in cell growth and differentiation. The association between HER2 positivity and poor prognosis, especially in breast cancer, has paved the way for the study of HER2 overexpression in gastric cancer. Overexpression of HER2 is found in approximately 10-30% of gastric cancers[2,5]. HER2 was found to be associated with male sex, intestinal type cancer, and moderate/good cell differentiation in gastric cancers[5]. The total gene expression analysis study conducted by targeting HER2 revealed that trastuzumab, a HER2 monoclonal antibody, may contribute favorably to survival in the treatment of gastric cancer[14]. The relationship between HER2 positivity and prognosis remains controversial[2,5]. HER2 was evaluated immunohistochemically in all specimens from the 114 patients included in our study. In 102 (89.5%) patients, the HER2IHC scores were 0 and 1+, indicating HER2-negativity. HER2 IHC scores of 2 (n=7; 6.1%) and 3 (n=5; 4.4%) were designated for the indicated number of patients. We were able to access the data of 99 patients whose *HER2/neu* gene expression status was evaluated using the fluorescence *in*

situ hybridization (FISH) method. HER2 overexpression was observed in 10 of 99 cases (10.1%). The findings obtained using both methods were consistent with the literature. The median survival time was 3.6 months in patients with HER2 overexpression (score 3), 18 months in patients with HER2 IHC scores of 2, and 22.1 months in HER2-negative patients. Similarly, median survival times were 11.4 and 22.9 months in patients with and without HER2 overexpression when the FISH method was used. The results obtained by both methods were quite similar. The median survival of patients with HER2 overexpression was markedly shorter, but no statistically significant difference was observed between both groups. Studies examining the relationship between HER2 and HIF-1α have shown that HER2 overexpression stimulates HIF-1α-dependent gene transcription not directly but in combination with other tumor-specific genetic and physiological changes[6]. It should be kept in mind that the fact that all HER2-positive patients in our study received targeted therapy may have led to the lack of a statistically significant difference in survival between groups with and without HER2 overexpression.

In mammalian cells, it is essential to maintain oxygen homeostasis to meet energy needs and sustain aerobic metabolism. In rapidly proliferating cancer cells, increased oxygen consumption and decreased oxygen transport and diffusion lead to hypoxia. Inadequate and chaotic vascularization also leads to severe deterioration of the oxygen balance in tumor cells[6,7]. It has been shown that HIF-1α plays an important role in the adaptation of tumor cells to these changes in oxygen concentrations and thus in tumor progression. HIF-1α realizes this adaptation by regulating many genes involved in the angiogenesis, glucose metabolism, cell proliferation, invasion, and metastasis pathways[17,18]. In the literature, HIF-1α overexpression has been reported in many organ cancers, including colorectal, breast, lung, ovarian, and pancreatic cancers. In most of these malignancies, HIF-1α is generally associated with poor prognosis[19]. Since gastric cancers still rank high in cancer mortality and are usually diagnosed at an advanced stage, the search for new treatment modalities and agents that will contribute to survival continues. HIF-1α is becoming one of the popular targets in the approach to gastric cancers[20,21].

Despite the advocacy of opposing views, in many studies, HIF-1α expression in a wide range of malignant tumor tissues, including gastric adenocarcinomas, has been associated with low survival and adverse clinicopathological factors[20-23]. In our study, the 1-year survival rates were 45.8% in HIF-1α positive, and 25.6% in the HIF-1α negative group with a statistically significant intergroup difference (p=0.02). Contrary to most studies cited in the literature, the 5-year survival rates were 36.9% and 22.3% in the HIF-1α positive, and negative groups with a statistically significant difference between groups. The existence of contradictory and sometimes conflicting results in the literature may be related to the fact that HIF-1α is a complex molecule that may have both apoptosis-inducing and inhibitory effects in cell metabolism, as well as stimulating or inhibiting cell proliferation[6,7]. In addition, pathways involved in tumor progression and induced pathways may be differentially stimulated by HIF-1α. It is thought that the level of hypoxia, presence of an oncogene, or homologs such as HIF-2α and HIF-3α may also be effective in the regulation of these pathways, which may affect HIF-1α expression levels in tumors[11]. In addition, interobserver differences in the evaluation of HIF-1α immunohistochemistry should be considered when analyzing different results. All of these data support the argument that further studies are needed to accept HIF-1α as a target molecule for determining prognosis and targeted therapies[24].

While HIF-1α expression was detected in 60% of the cases with extensive tumor necrosis, whereas 19.3% of the cases without necrosis were HIF-1α positive. Considering the role of HIF-1α in hypoxic conditions, this finding was considered to be compatible with the nature of the molecule, and the fact that this finding was not statistically significant may be related to the small number of cases with necrosis included in our study. In the regression analysis, the risk of death was 3.42 times higher in advanced tumors than in early-stage tumors.

Study Limitations

Our study has several limitations. The most important of these limitations is that the tissues used for immunohistochemical staining were obtained by the tissue microarray method, which suggests that our results may be affected by tumor heterogeneity. Second, there is no specific standardization for the immunohistochemical evaluation of HIF-1α expression. For example, Rohwer et al.[8] not only performed a quantitative evaluation of HIF1A expression in tumor cells but also divided them into groups to perform a qualitative evaluation, perhaps to eliminate the handicaps of heterogeneous staining. Because very small tumor areas were evaluated in our study, it was not possible to create ordinal groups. In addition, nuclear or nucleocytoplasmic

staining was used as the basis in the studies we referenced in our study. There is no consensus on any cut-off value for HIF-1α expression level. Under these conditions, comparing data obtained from different sources will not yield optimal results. Finally, the fact that patients who received neoadjuvant treatment were not allocated into different groups during patient selection may be a factor that may affect both HIF-1α expression and survival.

Conclusion

As a result, HIF1A and cancer-related studies have shown that HIF1A expression can have positive effects on cancer treatment and prognosis. HIF1A can also be used to improve survival in patients with gastric cancer, which is one of the most frequently seen and aggressive cancers. Therefore, new and comprehensive studies are needed in this regard.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee of the University of Health Sciences Turkey, İzmir Tepecik Education and Training Hospital (approval number: 2017/14-37, dated: 11.12.2017).

Informed Consent: Informed consent was obtained from all patients prior to surgery to allow the use of the surgical materials obtained for scientific purposes.

Acknowledgments

*An oral presentation titled the relationship of immunohistochemical HIF-1a expression to clinicopathological parameters in gastric carcinomas was performed at the 27th National Pathology Congress held in Antalya on 15-18 November 2017.

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*The English translation and editing of the article were done by Gökhan Kazancı.

Footnotes

Authorship Contributions

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

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