



Determining the relationship between cytokeratin expression and prognostic factors in human gastric cancer

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ABSTRACT

Determining the prognosis of gastric cancer is the most crucial step in the treatment process. Cytokeratins are intermediate filaments found in the intracellular structure of epithelial tissues. Recent researches have focused on determining the relationship between the expression of cytokeratins and the degree and prognosis of tumors. This study aimed to investigate the relationship between the incidence of cytokeratin-20 and cytokeratin-7 in patients with gastric carcinoma with factors influencing the prognosis. In this regard, the study was conducted cross-sectional. The expression of cytokeratin-20 and cytokeratin-7 was evaluated on 50 gastric adenocarcinoma specimens with different degrees of differentiation by the immunohistochemical method. We determined the relationship between the incidence of cytokeratin-20 and cytokeratin-7 with factors affecting the prognosis of patients, including the degree of differentiation of gastric cancer tissue, lymph node involvement, and the depth of tumor invasion. Data were statistically analyzed by Chi-square and Spearman tests. The results showed a statistically inverse relationship between the incidence of cytokeratin-20 and cytokeratin-7 with the degree of tissue differentiation and lymph node involvement in gastric cancer. Although there was a statistically significant relationship between the incidence of tissue invasion in gastric cancer and the incidence of cytokeratin-7, there was no association between the incidence of cytokeratin-20 and tissue invasion. In general, decreased cytokeratin-20 and cytokeratin-7 are associated with decreased tissue differentiation and increased lymph node involvement.

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Introduction

Gastrointestinal cancers, including colorectal and stomach cancers, are the most common cancers (1). It takes years for the early changes in stomach cancer to turn into invasive cancer (2). Early changes are rarely associated with symptoms and are often not detected in the early stages (3). Gastric cancers travel through the stomach wall to nearby tissues and organs or through arteries to the lymph nodes (4). They also involve other organs in more advanced stages through the bloodstream (5).

A vital issue after diagnosis is the prognosis and treatment of gastric cancer (6). The prediction of the tumor plays an essential role in the treatment design (5). Lymph node involvement, depth of tumor invasion, and tumor-specific markers determine the prognosis of gastric cancer. Cytokeratins are intermediate filaments found in the cytoskeletal component of epithelial cells (7). The subtype of

cytokeratins produced in the epithelial cell depends mainly on the epithelium. In cancerous tissues caused by epithelium (carcinomas), various profiles of cytokeratins are made (8). At present, researchers are trying to determine the relationship between cytokeratins and the type of malignancy, grade, and prognosis of tumors (8, 9).

Cytokeratin-20 is a medium-sized (46kDa) filamentous protein that first appeared on Merkel cells, uroepithelium, and gastric and intestinal epithelium. Cytokeratin-20 is a unique type of keratin type1 found in adenocarcinomas of the colon, stomach, pancreas, and biliary system. Research has shown that cytokeratin-20 can be used as a prognostic indicator. A study of 164 patients with gastric cancer found a significant association between the positive cytokeratin-20 index and gastric lavage secretions with venous invasion, lymphatic invasion, tumor size, pathological tumor type, peritoneal spread, and stage

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and depth (10).

A study by Kodera *et al.* (11) on 190 patients with gastric cancer also showed that the sensitivity of cytokeratin 20 to diagnose disseminated cases of gastric cancer was 93% with a specificity of 91%, Positive Predictive Value 70%, and Negative Predictive Value equal to 88%. Cytokeratin-7 is an intermediate filament weighing 54 kDa found in endocrine and transitional coatings but not squamous cell carcinoma. Without dissolving in water, this protein is involved in the formation of membranes and intercellular connections through attachment to the cytoskeleton. The staining pattern of cytokeratin-20 and cytokeratin-7 is different in tumors. This pattern has been used in the differential diagnosis of various tumors; For example, the staining pattern in colorectal malignancies is positive for cytokeratin-7, negative, and cytokeratin-20. In contrast, this pattern is different in gastric adenocarcinoma.

Early detection of gastric cancer in the early stages and identifying factors affecting the prognosis improve patients' quality of life (1). One of the issues emphasized today in the prediction of gastrointestinal cancers is the use of immunohistochemical tests to identify various molecules such as cytokeratins and tumor markers (12). Accordingly, due to the lack of studies in this field, this study was performed to determine the relationship between the incidence of cytokeratin-20 and cytokeratin-7 in patients with gastric carcinoma with factors affecting the prognosis of these patients.

Materials and methods

Study population

This research was a cross-sectional study with descriptive-analytical aspects. The statistical population included all patients referred to the hospital with a definitive diagnosis of primary gastric adenocarcinoma. Sampling was done non-randomly, and the sample size was 50 based on the number of available samples.

The study's inclusion criteria were the completeness of patients' files and the availability of invasive tumor tissue and lymph nodes. Accordingly, 50 samples that met the input criteria were included in the study. Each sample's demographic and clinical characteristics, including age and gender of patients, tumor depth,

number of lymph nodes involved, and degree of tumor differentiation, were recorded.

Tumor evaluation

From paraffin blocks of each sample, 3-micron sections were prepared and stained by the hematoxylin-eosin method. Using these sections, the degree of malignancy of each piece was determined and recorded as a well-differentiated tumor, a medium-differentiated tumor, and a poorly differentiated tumor.

Immunohistochemical method

For immunohistochemical analysis using labeled antibodies, this method stained the samples. Tissues molded with paraffin were cut to a thickness of 4 μ m. They were then passed through water and alcohol solution for 5 minutes, placed in a hot water bath, and put on a slide. The slides were placed in the oven at 60°C for 30 minutes in the next step. The samples were paraffinized and dehydrated through four containers of xylol, two containers of 100% alcohol, two containers of 96% and 75% alcohol, and then one container of distilled water, each for 5 to 10 minutes. They were then rinsed with 10% PBS (Phosphate buffered saline) two to three times. A solution of hydrogen peroxide and methanol in a ratio of (1 to 9) was used for 10 minutes to eliminate tissue peroxidation activity. Again, the slides were washed with 10% PBS, and then the slides were immersed in EDTA buffer solution at 120°C for 15 minutes. After we removed the slides from the autoclave, they were put at room temperature and rewashed with 10% PBS.

Samples with NovocastraLtd cytokeratin monoclonal antibody (England) (Mouse monoclonal antibody) were stained as follows:

First, two drops of serum blocking solution were poured on the slides and placed in the open air for 10 minutes. Then, two drops of the initial antibody solution were diluted 1/200 on the slides and kept at room temperature for 45 to 60 minutes. In the next step, the slides were washed with 10% PBS several times, and then two drops of biotin-containing secondary antibody solution were poured on the slides. After 10 minutes, the slides were rewashed with 10% PBS solution; then, two drops of conjugated enzyme solution were poured on the samples. Samples were washed with 10% PBS solution after 10 minutes.

Then, one drop of DAB chromogen solution with 1ml of distilled water was added to the samples. One drop of hematoxylin was used to create contrast and background color. At this stage, the slides were rinsed with distilled water for a few minutes and again passed through a container of distilled water and 75% alcohol for dewatering. The slides were then placed in 99% alcohol for 3 minutes and in two 100% alcohol for 5 minutes. For clarity, the samples were placed in four xylol containers each for 5 minutes. In the end, lamellas were placed on the samples. This study used small intestinal and cerebellar tissues as positive control and negative control, respectively. The presence or absence of cytokeratin-20 and cytokeratin-7 was determined using a light microscope (CH-2, Olympus, Japan) at 400x magnification. The total number of malignant cells and the total number of stained cells were counted. The percentage obtained by dividing the two was considered the percentage of staining; Above 5%, the criterion of the positive sample was evaluated.

Statistical analysis

The obtained data were statistically analyzed using SPSS software version 22 and Chi-square and Spearman statistical tests. The error of the first type was set at 0.05, and the range equal to or less was defined as the statistically significant range.

Results and discussion

Demographic and clinical results

Of the 50 gastric cancer specimens, 29 (58%) were male, and 21 (42%) were female. The mean age of patients was 62.27 years. 11 (22%) of the studied tumors had a good degree of differentiation, 19 (38%) had a moderate degree of differentiation, and 20 (40%) had a poor degree of differentiation. Lymph node involvement was present in 37 (74%) tumors. At the same time, 13 (26%) samples did not have lymph node involvement. There was complete involvement in 41 (82%) samples, including mucosal, submucosal, muscular, and serous layers. In 9 (18%) samples, the depth of invasion was limited to the muscle layer.

Immunohistochemical findings

Cells with positive cytokeratin index were seen with cytoplasmic brown staining.

A) Cytokeratin-20: A study of the frequency distribution of gastric cancer tissue differentiation based on the degree of positive cytokeratin-20 showed that regardless of the degree of tumor differentiation, 31 (62%) samples were cytokeratin-20 positive, and 19 (38%) samples of the cytokeratin-20 index was negative. Table 1 shows the frequency distribution of gastric cancer tissue differentiation based on the degree of cytokeratin-20 positivity.

Table 1. Frequency distribution of gastric cancer tissue differentiation based on the positive degree of cytokeratin-20

Type of differentiation	Positive cytokeratin-20 samples	Negative cytokeratin-20 samples
Good	8 (16%)	3 (6%)
Medium	14 (28%)	5 (10%)
Weak	9 (18%)	11 (22%)
Total	31 (62%)	19 (38%)

Examination of the frequency distribution of lymph node involvement in gastric cancer based on the degree of positive cytokeratin-20 showed that the incidence of cytokeratin-20 in samples with lymph node involvement is the highest (46%). Table 2 shows the frequency distribution of lymph node involvement in gastric cancer based on the positive degree of cytokeratin-20.

Table 2. Frequency distribution of lymph node involvement in gastric cancer based on the positive degree of cytokeratin-20

Lymph node involvement	Positive cytokeratin-20 samples	Negative cytokeratin-20 samples
Having	23 (46%)	15 (30%)
Not having	8 (16%)	4 (8%)
Total	31 (62%)	19 (38%)

Examination of the frequency distribution of samples based on the degree of invasion showed that the highest number of 24 samples belonged to complete mucosal involvement with cytokeratin-20 positive index. In contrast, the lowest number of samples in cases of incomplete layer involvement is seen in cytokeratin negative samples. Table 3 shows the frequency distribution of tissue invasion in gastric cancer based on the degree of cytokeratin-20 positivity.

Table 3. Frequency distribution of tissue involvement in gastric cancer based on the positive degree of cytokeratin-20

Tissue involvement rate	Positive cytokeratin-20 samples	Negative cytokeratin-20 samples
Involvement of mucosal, submucosal, muscular, and serous layers	24 (48%)	16 (32%)
Incomplete involvement of layers	7 (14%)	3 (6%)
Total	31 (62%)	19 (38%)

B) Cytokeratin-7: The frequency distribution of the cytokeratin-7 index in terms of tissue differentiation, lymph node involvement, and tissue involvement in the 31 samples was as follows.

Examination of the frequency distribution of gastric cancer tissue differentiation based on the degree of cytokeratin-7 positivity showed that regardless of the degree of tumor differentiation in 14 (28%) samples, cytokeratin-7 was positive. In 36 (72%) samples, the cytokeratin-7 index was negative. Table 4 shows the frequency distribution of gastric cancer tissue differentiation based on the positive degree of cytokeratin-7.

Table 4. Frequency distribution of gastric cancer tissue differentiation based on the positive degree of cytokeratin-7

Type of differentiation	Positive cytokeratin-7 samples	Negative cytokeratin-7 samples
Good	11 (22%)	8 (16%)
Medium	3 (6%)	16 (32%)
Weak	1 (2%)	12 (24%)
Total	14 (28%)	36 (72%)

Examination of the frequency distribution of lymph node involvement in gastric cancer based on the degree of positive cytokeratin-7 showed that in 27 (54%) samples, despite the involvement of lymph nodes, the cytokeratin-7 index was not evident. It was the highest number. Table 5 shows the frequency distribution of lymph node involvement in gastric cancer based on the positive degree of cytokeratin-7.

Table 5. Frequency distribution of lymph node involvement in gastric cancer based on the positive degree of cytokeratin-7

Lymph node involvement	Positive cytokeratin-7 samples	Negative cytokeratin-7 samples
Having	10 (20%)	27 (54%)
Not having	4 (8%)	9 (18%)
Total	14 (28%)	36 (72%)

Examination of the frequency distribution of samples based on the degree of invasion showed that the highest number of samples belonged to cases with negative cytokeratin-7 index with complete involvement of four layers of mucosa, submucosa, muscle, and serosa that 32 (64%) samples were in this group. Table 6 shows the frequency distribution of tissue involvement in gastric cancer based on the degree of cytokeratin-7 positivity.

Table 6. Frequency distribution of tissue involvement in gastric cancer based on positive degree of cytokeratin-7

Tissue involvement rate	Positive cytokeratin-7 samples	Negative cytokeratin-7 samples
Involvement of mucosal, submucosal, muscular, and serous layers	8 (16%)	32 (64%)
Incomplete involvement of layers	6 (12%)	3 (6%)
Total	14 (28%)	36 (72%)

The study of the relationship between the incidence of cytokeratin-7 and the studied variables showed a statistically significant relationship between the degree of tissue differentiation of gastric cancer and the level of cytokeratin-7 ($P = 0.001$). A statistically significant association was found between lymphatic involvement and cytokeratin-7 levels ($P = 0.004$). There was a statistically significant relationship between the rate of tissue invasion in gastric cancer and cytokeratin-7 levels ($P = 0.0002$).

According to the findings of this study, there is a statistically inverse relationship between the incidence of cytokeratin-7 and the degree of tissue differentiation, lymph node involvement, and tissue invasion in gastric cancer.

The present study's findings on 50 samples of gastric adenocarcinoma showed a statistically inverse relationship between the incidence of cytokeratin-20 and cytokeratin-7 with the degree of tissue differentiation and lymph node involvement in gastric cancer. Although there was a statistically significant relationship between the rate of tissue invasion in gastric cancer and cytokeratin-7 levels, there was no correlation between the incidence of cytokeratin-20 and tissue invasion.

Preliminary studies have shown that the incidence of cytokeratin-20 in gastric cancer is of diagnostic value in diagnosing disseminated cases of gastric

cancer, the pathological type of the tumor, and its spread to the peritoneum (13, 14).

Al-Maghrabi *et al.* (15) examined the incidence of cytokeratin-20 and cytokeratin-7 about the factors that cause different patterns of these indicators in gastric and colorectal carcinomas. They found that cytokeratin-7 was positive in 71% of cases and cytokeratin-20 in 41% of the samples regarding gastric carcinomas. The expression of cytokeratin-20 and cytokeratin-7 in gastric carcinoma was not associated with the degree of tumor differentiation. The study results showed that the pattern of expression of cytokeratin-20 and cytokeratin-7 in gastric carcinoma is very different and contradictory. In various studies, the incidence of cytokeratin-7 has been reported from 10 to 70%, and the incidence of cytokeratin-20, between 30 and 50%.

In the present study, the incidence of cytokeratin-20 was 62%, and the incidence of cytokeratin-7 was 28%. This finding is similar to the results of previous studies. The difference in the range of indicators is due to the number of samples and their grouping.

Comparing data from 29 studies, Tot (16) examined the expression patterns of cytokeratin-20 and cytokeratin-7 regarding primary tumor location and metastasis. This study showed that the incidence of cytokeratin-20 was in primary gastric adenocarcinoma, and its metastasis varies. This finding is consistent with the results of this study. The present study showed that the levels of cytokeratin-7 and cytokeratin-20 are entirely different from the rate of tissue invasion in gastric cancer. The incidence of cytokeratin-20 is not associated with tissue invasion.

You *et al.* (13) studied the expression of cytokeratin-20 and cytokeratin-7 in terms of clinical and pathological components. The findings of this study have shown that the pattern of occurrence of these two indicators is variable in terms of the studied components. The incidence of cytokeratin-20 and cytokeratin-7 was higher in the samples with good differentiation and lowered in the samples with poor differentiation. The findings of this study are consistent with the present study results. These findings suggest that cells lose the ability to produce cytokeratin in poorly differentiated tumors.

Previous studies have shown that the expression of cytokeratin-7 has little value in the differential diagnosis of metastatic carcinoma (17-19). At the

same time, in some tumors such as prostate and hepatocellular carcinoma, the absence of cytokeratin-7 is of more excellent diagnostic value (20-22). Cytokeratin-20 occurs mainly in colorectal carcinomas and one-third of gastric adenocarcinomas (23-25).

This study showed that the depth of tissue invasion in gastric cancer is associated with the incidence of cytokeratin-7. But there was no association between the incidence of cytokeratin-20 and tissue invasion. Because the expression patterns of cytokeratin-20 and cytokeratin-7 in different carcinomas and their metastatic tissues are different and sometimes unique, the staining pattern of these two indicators can be used in the differential diagnosis of epithelial tumors. The study's limitations were Lack of access to complete clinical staging, and reduction of the number of samples to match them with the inclusion criteria. Due to the findings of the research and some other studies on the relationship between the incidence of cytokeratin index and prognostic factors in gastric adenocarcinoma, it is recommended that future studies with more samples and follow-up patients evaluate the exact effect of this factor on prognosis.

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Interest conflict

None.

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