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Evaluating STC2 gene RNA in peripheral blood serum of gastric cancer patients

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ABSTRACT

Cancer is one of the leading causes of death in the world. Invasive microscopic and endoscopic methods are not suitable for routine screening of gastric cancer. Therefore, the need for biomarkers that can detect quickly, efficiently, and with high sensitivity in the early stages of cancer is strongly felt. Stanniocalcin plays a role in regulating calcium and phosphorus in the body. In addition, it plays a role in neuron cell differentiation, angiogenesis, wound healing, fertility and embryo development, and cancer. This study investigated the level of expression of the Stanniocalcin 2 (STC2) gene in the serum of patients with gastric cancer. This case-control study was conducted on the samples of 60 patients with gastric cancer (cases) and 60 healthy individuals (controls). Peripheral blood samples from gastric cancer patients and volunteers as control groups were collected in tubes containing EDTA anticoagulant and were immediately subjected to serum separation. After centrifugation, serum RNA was extracted, and after cDNA synthesis, STC2 gene serum RNA level was measured by the Taq Man method and Real-time PCR using specific primers and probes. Then, the results of serum evaluations and clinicopathological information of patients and control group were collected along with the information obtained from reviewing patients' files and demographic findings, regulatory tables, and related charts. SPSS22 software was used to analyze descriptive data. According to the study results, the high expression of the STC2 gene was 31 cases in the case group and 13 cases in the control group. However, there was a significant relationship between the high expression of the STC2 gene and gastric cancer ($p = 0.0001$). However, there was no significant relationship between the gender of patients and high expression of the STC2 gene. However, the age of 35% of the patients was more than 65 years, and there was a significant relationship between the age of the patients and the high expression of the STC2 gene ($P = 0.028$). Although there was no significant relationship between the anatomical location of the cancer and the subtypes of the cancer and the high expression of the STC2 gene, there was a significant relationship between the degree of cancer differentiation and the high expression of STC2 gene ($P<0.05$). In general, STC2 can be used as a biomarker to determine the border and margins of the tumor. Analysis of STC2 gene expression during surgery can reduce surgical error in tumor removal and increase the success of surgery for tumor removal.

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Introduction

Although the incidence of stomach cancer has decreased worldwide, especially in developed countries such as the United States, in recent decades, its incidence has increased in China, which is particularly significant in Western China (1). According to the latest research conducted in 2020, in China, the rate of stomach cancer is 3.9 per 100,000, the third most common cancer in the country in both men and women (2).

Stomach cancer is a multi-factorial disease and has a multi-stage occurrence process. Among the factors that cause cancer, we can mention many infectious, environmental, genetic, and epigenetic factors, nutrition, lifestyle, age, and race (3). In more than half of the advanced cases, no symptoms are observed in people with this disease, and it is diagnosed when it has entered the advanced stage, and unfortunately, many of these people die after a short period. Meanwhile, the quick and timely diagnosis of this disease can increase the 5-year survival rate of affected people by 60% (1). Microscopic and endoscopic methods that are used today to diagnose stomach cancer are invasive methods that are not suitable for routine screening. Therefore, the need for biomarkers that can detect quickly and with high sensitivity in the early stages is strongly felt (4).

Stanniocalcin (STC) is a glycoprotein hormone named after the cartilaginous substance found in the endocrine gland of fish kidneys (5). STC prevents the entry of calcium ions into the target tissue by having anti-calcium properties. Increased expression of STCs is widely found in cancers such as the kidney, heart, pancreas, and spleen (6). The human autologous of this gene in humans is called Staniocalcin-1, which has been identified in immortal cells and is one of the most prominent characteristics of cancer cells (7). The paralog of the Stanniocalcin gene,

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i.e., Staniocalcin-2, has been identified by searching the related sequence in the Sequence Tag EST (Expressed) database. Although STC2 and STC1 are not expressed at the same time, both are expressed in different types of tissues, including endocrine glands and hormone-sensitive organs (8). The ovaries seem to have the highest amount of STC expression during pregnancy and lactation. STC1 is also found in the ductal epithelium of the breast. In recent years, evidence shows that the expression of STC changes in different types of cancers in humans (9). DNA microarray analysis shows that STC1 is increased in primary hepatocellular carcinoma (5). Also, STC1 is decreased in ovarian cancer cells compared to healthy epithelial cells (7, 10). An anonymous cDNA fragment study shows that STC1 is reduced in breast cancer cell lines. However, STC2 and STC1 are both expressed in ER-positive breast cancer. Nowadays, efforts are being made to use STC1 and STC2 expression in diagnosing and classifying breast cancer. The role of STC1 as a prognostic factor has been studied in many cancers, including gastric cancer (11). Therefore, the present study was conducted to investigate the level of STC2 gene expression in the serum of patients with gastric cancer.

Materials and Methods

Study method

This study is a case-control study that was conducted for one year. Sample size In this study, 60 patients with gastric cancer referred to the hospital, who were given a definitive diagnosis based on pathology criteria and underwent surgery, were considered the patient group. Also, 60 healthy people with no family history of cancer were included in the study as a control group.

Inclusion criteria include all patients with gastric cancer whom the pathology laboratory confirmed. The patient gives informed consent for the study. Patients with a history of chemotherapy or radiotherapy, simultaneous presence of other malignancies, and lymphoma were excluded from the study.

Peripheral blood samples were collected from patients with early gastric cancer and volunteers in tubes containing EDTA anticoagulant. The samples were immediately subjected to serum separation. To separate the serum, after spending the necessary time to clot the collected blood, we centrifuged the sample for 10 minutes at a speed of 1000xg. Then the separated serum was centrifuged for 10 minutes at a temperature of 2 degrees Celsius and a speed of 3500x to sediment the cells in it. The supernatant serum was passed through a 0.2um filter and kept to ensure the absence of cells in it. Imitrogen Trizol LS solution (Life Technologies, Carlsbad, CA, USA) was used to extract RNA from serum samples.

Real-time PCR method

Total RNA was extracted from the serum of control and patient samples, and then cDNA synthesis was performed using the Omniscript Reverse Transcriptase enzyme produced by Qiagen, Germany, and on the template strand. The concentration and quality of the extracted RNA were measured by reading the absorption intensity (OD) using a spectrophotometer (Nanodrop). The quantitative PCR method or Real-time PCR based on TaqMan primers and probes is a particular method to count the number of copies of RNA or DNA in a sample (12) (Table 1).

TaqMan probes are hydrolytic probes designed to increase the specificity of quantitative PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as a reference gene, and by taking into account the average CT values related to the target genes and their difference, we obtained the ΔΔCT level.

Statistical analysis

The serum RNA level of the STC2 gene was measured. The results of serum evaluations and the clinicopathological information of the patients and the control group were obtained using the checklists filled by the studied samples. The results were analyzed using t-test and Fisher exact test statistical methods by comparing delta CT obtained from patients and healthy volunteers. After collecting the data obtained from examining the patients' files and demographic findings and setting up the relevant tables and graphs, central indices (mean, median, etc.) and dispersion indices, standard deviation, variance, etc., were used to analyze the descriptive information. SPSS 22 software was used for data analysis. Due to the lack of standard data distribution, the Spearman correlation coefficient and Mann-Whitney U test and Knuskal-Wallis test were used for analysis. A level of less than 0.05 was considered significant.

Results

37 patients were male, and 23 patients were female. 34 people from the control group were male, and 26 from the control group were female. The average age of gastric cancer patients was 61.16 years with an age range of 72-28 years, and the average age of healthy people (control group) was 60.41 years with an age range of 36-74 years. Twenty-one people from the samples were over 65 years old, and 39 were less than 65 years old. According to the study results, 31 patients in the patient group and 13 people in the control group had high STC2 gene expression.

Although there was a significant relationship between the high expression of the STC2 gene and gastric cancer (p=0.0001), there was no significant relationship between the gender of the patients and the high expression of the gene (p=0.68). However, the age of 35% of the patients

Table 1. Primer sequences and product length of GADPH and STC2 in real-time PCR.

Gene	Primer Sequence (5'-3')	Product length
GAPDH	Forward: GACTTATCCGATGATTCTTCAGCG	216 bp
	Reverse: TGGCTGACTGCTCTTGTGAGCAT	
	Probe: FAM-CGAATCCAACTCTGATCAGTCCGTAGT-TA MRA-1	
STC2	Forward: CGGATGATTCAGTGCGATCGA	125 bp
	Reverse: CGATGTCGGTGTTATCATGGCGA	
	Probe: FAM-CGATTCGATGCATGGCATAATGCCAGTGA-TA MRA-1	

was more than 65 years, and there was a significant relationship between the age of the patients and the high expression of the STC2 gene (P = 0.028).

Table 2 shows that gastric cancer's most common anatomical location was in the stomach trunk (71.66%).

Table 3 shows no significant relationship between the anatomical location of cancer and high expression of the STC2 gene (P=0.28).

Table 4 shows that the high expression of STC2 in diffuse-type adenocarcinoma was 54.83%, and in intestinal-type adenocarcinoma was 45.17%. There was no significant relationship between cancer subtypes and high STC2 gene expression (P = 0.13).

Table 5 shows the relationship between the degrees of differentiation of cancer with high expression of STC2. There is a significant relationship between the degree of cancer differentiation and the high expression of the STC2 gene (P = 0.29).

Discussion

According to the results of the present study, the high expression of the STC2 gene was 31 cases in the patient group and 13 cases in the control group. There was a significant relationship between the high expression of the STC2 gene and stomach cancer (P=0.0001). In a study conducted by Fang *et al*. (10), 83 patients with gastric cancer treated with radical resection were included. Immunohistochemistry was used to detect STC protein in tumors and adjacent normal tissues. The serum level of STC was determined by enzyme-linked immunosorbent assay (ELISA), and a receiver operating characteristic (ROC) curve was constructed to describe the diagnostic specificity and sensitivity. They concluded that STC1 and STC2 protein expression increased in gastric cancer tissues compared to normal tissue. Our study is also in line with the above study. Another study in Japan found a significant relationship between the mRNA expression of different genes, including the STC2 gene, in the peripheral blood samples of gastric cancer patients before surgery, patients with recurrence, and the control group (12). In our study, the high expression of the STC2 gene was 31 cases in the patient group and 13 cases in the control group. There was a significant relationship between high expression of the STC2 gene and gastric cancer (P=0.0001). In addition, high and moderate levels of STC1 protein were significantly related to lymph node metastasis and a low chance of patient survival (3 years). The serum STCI and STC2 expression in patients with GC was much higher than in patients with benign gastric disease, which decreased 7 to 10 days after surgery. The sensitivity of serum STC protein was also superior to CEA and CA19-9. Serum STC1 and STC2 may serve as promising tumor biomarkers for the diagnosis and prognosis of gastric cancer (10).

Most tissue samples were average regarding cancer differentiation (45.16%). There was no significant relationship between cancer differentiation and high STC2 gene expression (p = 0.29). For most patients (23 people), their TNM stage was T3; there was a significant relationship between cancer classification and high expression of the STC2 gene (p = 0.001). TNM stands for Tumor (T), Node (N), and Metastasis (M), and doctors pay attention to these three factors to determine the stage of cancer (13). A study by Arigami *et al*. (12) used a quantitative PCR test to evaluate STC2 mRNA expression in 4 gastric medullary cells and in blood samples of 93 patients with gastric cancer and healthy volunteers. They concluded that the number of STC2 mRNA copies in gastric cancer cells and in the blood of patients with this cancer was higher than that of healthy volunteers (p = 0.02 and 0.01). STC2 gene expression was positive in 43 patients (46.2%) out of 93 patients with gastric cancer. Its expression has a significant relationship with age, depth of tumor invasion, lymph node metastasis, stage, and venous invasion (p=0.023, p = 0.007, p = 0.045, p = 0.035, and p = 0.027). The 5-year survival rate was significantly lower in patients with STC2 expression than those without STC2 expression (p = 0.014) (12). Our results show that STC2 can be a useful molecular biomarker for predicting tumor progression by monitoring CTC in gastric cancer patients. The above study is also in line with our study.

Table 2. Frequency distribution of anatomical site for gastric cancer.

Lesion Location	Frequency	Percent
Cardia	26	43.33
Stomach trunk	43	71.66
Antrum	28	46.66
Diffuse-type	21	35
Total	60	100

Table 3. Frequency correlation of anatomical site for cancer with high expression of STC2 gene.

Lesion Location	Frequency	Percent	P-value
Cardia	7	22.58	0.28
Stomach trunk	12	38.71	
Antrum	8	25.81	
Diffuse-type	4	12.90	
Total	31	100	

Table 4. Frequency correlation of cancer subtypes with high expression of STC2.

Lesion Location	Frequency	Percent	P-value
Intestinal	17	54.83	0.13
Diffuse-type	14	45.17	
Total	31	100	

Table 5. The relationship between the frequency of cancer differentiation and the high expression of SCT2.

Cancer differentiation	Frequency	Percent	P-value
Good	11	35.48	0.29
Medium	14	45.16	
Weak	6	19.36	
Total	31	100	

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