CLINICAL VALUE OF COMBINED DETECTION OF CA199, CEA AND HEAT SHOCK PROTEIN IN THE DIAGNOSIS OF RECTAL CANCER

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ABSTRACT

Objective: *To analyze the clinical value of combined detection of carbohydrate antigen-199 (CA199), carcinoembryonic antigen (CEA), and heat shock protein 90 α (Hsp90α) in the diagnosis of rectal cancer.*

Methods: *142 patients with rectal cancer treated in our hospital from January 2019 to 2020 were selected as the rectal cancer group, 56 patients with benign proctitis were selected as the benign lesion group, and 40 healthy people in the physical examination center of our hospital were selected as the healthy control group. According to Dukes stage, rectal cancer patients were divided into four groups: A, B, C, and D. According to the degree of differentiation, the patients were divided into four groups: high differentiation group, medium differentiation group, low differentiation group and undifferentiated group. The levels of CA199 and CEA were detected by chemiluminescence method, and serum Hsp90 α levels were detected by enzyme-linked immunosorbent assay (ELISA). The serum CEA, CA199, and Hsp90α levels were compared among all groups. The influencing factors of rectal cancer development were analyzed by univariate and multivariate Cox regression. ROC curve was used to analyze the value of CEA, CA199, and Hsp90α levels in the diagnosis of rectal cancer.*

Results: *The serum levels of CEA, CA199, and Hsp90α in benign lesion group and rectal cancer group were significantly higher than those in healthy control group. Serum CEA, CA199, and Hsp90 α levels in rectal cancer group were significantly higher than those in benign lesion group (P<0.05). With the progress of Dukes staging, serum CEA, CA199, and Hsp90α levels in patients with rectal cancer were gradually increased (P<0.05). Serum CEA, CA199, and Hsp90α levels in patients with poorly differentiated and undifferentiated rectal cancer were significantly higher than those in patients with moderately differentiated and well-differentiated rectal cancer (P<0.05). By univariate and multivariate Cox regression analysis, CEA, CA199, and Hsp90α were the influencing factors for rectal cancer progression (P<0.05). ROC curve analysis showed that the AUC of CEA in the diagnosis of rectal cancer was 0.714, the sensitivity was 75.62%, and the specificity was 70.14%; the AUC of CA199 in the diagnosis of rectal cancer was 0.768, the sensitivity was 78.64%, and the specificity was 73.54%; the AUC of Hsp90α in the diagnosis of rectal cancer was 0.809, the sensitivity was 83.64%, the specificity was 81.07%; the AUC of the combination of CEA and CA199 was 0.876, the sensitivity was 89.64%, and the specificity was 86.37%.*

Conclusion: *Serum levels of CEA, CA199, and Hsp90α are high in patients with rectal cancer, and they are closely related to Dukes stage and differentiation degree. They are influencing factors in the development and progression of rectal cancer, and they play a certain role in the diagnosis of rectal cancer. The combination of the three levels has the highest value and can be widely used in clinical practice.*

Keywords: *CA199, CEA, heat shock protein, detection, joint diagnosis, rectal cancer, clinical value.*

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Introduction

Rectal cancer refers to cancer found from the dentate line to the rectum-sigmoid colon junction, and it is the third most common tumor in the world, after lung cancer and breast cancer[(1)]. With rapid economic development in recent years, the living and eating habits and structure of residents have changed, and the incidence of rectal cancer has been increasing yearly, seriously endangering human life and health[(2)]. Studies have found that the 5-year survival rate of early rectal cancer is more than 90%, while that of late rectal cancer is less than 10%. Therefore, early diagnosis and early treatment of rectal cancer is of great significance for effective treatment of patients, improvement of

patients' quality of life, and extension of patients' life expectancy[3]. Carcinoantigen-199 (CA199) and Carcinoembryonic antigen (CEA) are widely recognized tumor markers for the diagnosis of colorectal cancer, but they are not sensitive enough for early diagnosis of colorectal cancer. Misdiagnosis often occurs, which affects the follow-up treatment of patients[4]. Heat shock proteins (HSP), a family of cellular stress proteins, are highly conserved evolutionarily and widely present in various cells[5].

Heat shock protein 90a (Hsp90α) is one of the subtypes of HSP, which is abnormally expressed in gastric cancer, pancreatic cancer, lung cancer, and nephroblastoma; it is closely related to the occurrence and development of tumors[6]. Recent studies have found that serum Hsp90α levels in patients with rectal cancer show an abnormal trend of increase, which may be associated with rectal cancer[7]. In this study, 142 cases of patients with rectal cancer treated in our hospital from January 2019 to 2020 were selected for an observation that aimed to analyze the clinical value of serum levels of CA199, CEA, and heat shock protein for the combined diagnosis of rectal cancer.

Materials and methods

General information

A total of 142 patients with rectal cancer treated in our hospital from January 2019 to 2020 were selected as the rectal cancer group.

Inclusion criteria were as follows:

• All patients were confirmed to have been diagnosed with rectal cancer by pathology test;

• The patient had not previously visited the doctor and no other treatment was given;

• Patients and their family members received information and signed the informed consent. During the same period, 56 patients with benign proctitis were selected as the benign lesion group.

Inclusion criteria were as follows:

• Patients had been diagnosed with benign proctitis;

• The patient had not previously visited the doctor and no other treatment was given;

• Patients and their family members received information and signed the informed consent.

A total of 40 healthy people were selected as the control group.

Inclusion criteria were as follows:

• Patients had no diseases related to the gastrointestinal system;

• Patients and their family members received information and signed the informed consent.

Exclusion criteria for the three groups were:

• Serious dysfunction of heart, liver, kidney, or other vital organs;

• Complications involving other malignant tumors;

• Systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, or other autoimmune diseases;

• Severe asthma or endometrial polyps;

• Refusal to participate in the experiment or termination of participation the experiment for other reasons.

In the rectal cancer group, there were 142 patients, 74 males and 68 females, with an average age of 45.08±9.69 years, and an average Body Mass Index (BMI; measure of weight in kilograms divided by the height in meters squared) of 20.09±1.05 Kg/m^2. In terms of Dukes stage, 50 cases were stage A, 37 cases were stage B, 29 cases were stage C, and 26 cases were stage D. There were 49 cases of high-grade type, 40 cases of medium-differentiation type, 28 cases of low-differentiation type, and 25 cases of undifferentiated type. In the benign lesion group, there were 56 patients, including 30 males and 26 females, with an average age of 45.11±9.56 years and an average BMI of 20.15±1.02.

In the control group, there were 40 patients, including 22 males and 18 females, with an average age of 45.06±9.78 years and an average BMI of 20.05±0.98. There was no significant difference in age, sex, or BMI among all groups in the rectal cancer group (P>0.05).

Observation indexes

Serum detection

5 ml of fasting venous blood was collected from patients with rectal cancer and benign proctitis within 24 h of admission, and 5ml of fasting venous blood was collected from healthy people during physical examination. The blood was kept at room temperature for 20 min and centrifugated at 3000 r/min for 10 min. The serum was carefully separated and refrigerated at -70°C for later use to avoid repeated freezing and thawing. The levels of CA199 and CEA in serum were detected by chemiluminescence assay, and the levels of serum Hsp90α were detected by enzyme-linked immunosorbent assay (ELISA). The levels of serum CEA, CA199, and Hsp90α in each group were compared.

Statistical methods

The SPSS20.0 software package was used for statistical analysis of data in this study. Comparison of all measurement data was expressed as $\bar{x}\pm s$, and a t-test was used for comparison between groups. Enumeration data were expressed as percentages, and a χ^2 test was used for comparison between groups. Univariate and multivariate Cox regressions were used to analyze the factors influencing the occurrence and development of rectal cancer. ROC curve was used to analyze the value of CEA, CA199, and Hsp90α in the diagnosis of rectal cancer. The statistical results were considered statistically significant if P<0.05.

Results

Comparison of serum levels of CEA, CA199, and Hsp90 α among the three groups

The levels of serum CEA, CA199, and Hsp90α in benign disease group and rectal cancer group were significantly higher than those in healthy control group, and the levels of serum CEA, CA199, and Hsp90 α in rectal cancer group were significantly higher (P<0.05) than those in benign disease group. See Table 1.

Group	n	CEA (ng/ml)	CA199 (U/ml)	HSP90 α (ng/ml)
Healthy control group	40	2.49±2.08	16.36±11.45	40.16±20.15
Benign disease group	56	3.94±2.45[a]	23.49±11.02[a]	66.38±20.15[a]
Rectal cancer group	142	30.85±10.54[ab]	104.58±12.46[ab]	120.64±74.15[ab]
F		315.580	1416.030	37.470
t		<0.001	<0.001	<0.001

Table 1: Comparison of serum levels of CEA, CA199, and Hsp90α among the three groups ($\bar{x}\pm s$).
[a]Compared to healthy control group, P<0.05. [b]Compared to the benign lesion group, P<0.05.

Comparison of serum levels of CEA, CA199, and Hsp90α in rectal cancer patients with different Dukes stages

With the progression of Dukes stage, the levels of serum CEA, CA199, and Hsp90α in rectal cancer patients increased gradually, and the difference was statistically significant (P<0.05), as shown in Table 2.

Comparison of serum CEA, CA199, and Hsp90α levels in colorectal cancer patients with different degrees of differentiation

Serum levels of CEA, CA199, and Hsp90α in patients with low-and undifferentiated rectal cancer were significantly higher (P<0.05) than those in patients with moderate-and high-differentiated rectal cancer. See Table 3.

Dukes stage	n	CEA (ng/ml)	CA199(U/ml)	HSP90α (ng/ml)
A	50	6.89±3.46	28.46±20.15	71.52±54.12
B	37	9.52±4.19[a]	30.46±25.16[a]	105.34±74.16[a]
C	29	25.16±7.46[ab]	45.37±25.94[ab]	142.64±90.15[ab]
D	26	30.78±10.16[abc]	62.58±42.15[abc]	188.94±90.15[abc]
F		118.500	10.370	16.120
t		<0.001	<0.001	<0.001

Table 2: Comparison of serum levels of CEA, CA199, and Hsp90α in rectal cancer patients with different dukes stages ($\bar{x}\pm s$).
[a]Compared to group A, P<0.05. [b]Compared to group B, P<0.05. [c]Compared to group C, P<0.05.

Degrees of differentiation	n	CEA (ng/ml)	CA199(U/ml)	HSP90α (ng/ml)
High differentiated	49	6.85±4.15	29.68±24.15	88.64±51.26
Moderate	40	13.59±9.64[a]	42.64±15.48[a]	108.35±35.18[a]
Low	28	25.64±20.15[ab]	55.68±24.61[ab]	131.54±45.64[ab]
Undifferentiated	25	35.15±21.15[abc]	69.94±30.14[abc]	168.95±55.64[abc]
F		27.960	18.540	26.560
t		<0.001	<0.001	<0.001

Table 3: Comparison of serum levels of CEA, CA199, and Hsp90α in colorectal cancer patients with different degrees of differentiation ($\bar{x}\pm s$).
[a]Compared to group A, P<0.05. [b]Compared to group B, P<0.05. [c]Compared to group C, P<0.05.

Analysis of factors influencing occurrence and progression of rectal cancer

Univariate and multivariate Cox regression analyses showed that CEA, CA199, and Hsp90α were all influential factors for the development of rectal cancer (P<0.05). See Table 4.

Factors	Univariate analysis			Multivariate analysis		
	95% CI	HR value	*P* value	*95% CI*	HR value	*P* value
CEA	1.426-12.145	4.241	0.012	1.008-9.124	3.041	0.009
CA199	1.581-3.795	2.561	0.003	1.214-3.436	1.985	0.041
HSP90α	1.496-6.651	3.412	0.015	0.658-5.402	1.748	0.001

Table 4: Analysis of the factors influencing the occurrence and progression of rectal cancer.

Value analysis of CEA, CA199, and Hsp90α in the diagnosis of rectal cancer

ROC curve analysis showed that the AUC, sensitivity, and specificity of CEA in the diagnosis

of rectal cancer were 0.714, 75.62%, and 70.14%, respectively. The AUC, sensitivity, and specificity of CA199 in the diagnosis of rectal cancer were 0.768, 78.64%, and 73.54%, respectively. The AUC, sensitivity, and specificity of Hsp90α in the diagnosis of rectal cancer were 0.809, 83.64%, and 81.07%, respectively. The AUC, sensitivity, and specificity of combined diagnosis was 0.876, 89.64%, 86.37%, respectively. As shown in Table 5.

Indicator	AUC	95% CI	*P* value	Sensitivity	Specificity
CEA	0.714	0.669-0.768	0.001	75.62%	70.14%
CA199	0.768	0.714-0.816	0.005	78.64%	73.54%
HSP90α	0.809	0.761-0.863	0.014	83.64%	81.07%
Combined	0.876	0.824-0.927	0.001	89.64%	86.37%

Table 5: Value analysis of CEA, CA199, and HSP90α in the diagnosis of rectal cancer.

Discussion

Colorectal cancer is one of the most common tumors of the digestive tract. As China's lifestyle and dietary habits become more internationalized, the incidence and mortality of rectal cancer have gradually increased, becoming one of the malignant tumors most affecting the health of Chinese people and placing a heavy economic burden on China[8].

Tumor markers are either produced by cancerous cells, or they belong to a class of substances produced by the body in response to tumor cells. Tumor markers play an important role in the differentiation of benign and malignant tumors, tumor auxiliary diagnosis, and evaluation of efficacy and prognosis[9]. CEA is one of the tumor markers of rectal cancer in clinic, but its level is also increased in patients with pancreatic cancer, gastric cancer, lung cancer, urethral cancer, and ovarian cancer[10]. CA199 is a mucin-type glycoprotein tumor marker, which is most often abnormally expressed in gastrointestinal tumors such as gastric cancer, rectal cancer, and pancreatic cancer, and is a good indicator for the detection of gastrointestinal malignancies. Recent studies have shown that the positive rate of CA199 in colorectal cancer is about 18.00%[11]. The high expression of HSP in malignant tumors can enhance the anti-apoptotic property of tumor cells, protect tumor cells, and also play a role in tumor immunity and tumor drug resistance[12].

Under normal circumstances, Hsp90α is maintained at a low level. The expression of Hsp90α increases to varying degrees when the cell is stimulated by the external environment, so as to ensure the correct expression of intracellular proteins and to achieve the function of stabilizing cell function and adaptation to external stress[13]. In tumor cells, Hsp90α can bind to apoptosis inhibitors, induce cell transformation into S phase and promote cell proliferation. To ensure the growth of tumor cells, the content of Hsp90α in cells was significantly increased[14]. In this experiment, the levels of serum CEA, CA199, and Hsp90α in the benign lesion group and the rectal cancer group were significantly higher than those in the healthy control group, and the levels of serum CEA, CA199, and Hsp90α in the rectal cancer group were significantly higher than those in the benign lesion group. Serum levels of CEA, CA199, and Hsp90α increased gradually in patients with rectal cancer, and the levels of CEA, CA199, and Hsp90α in patients with poorly differentiated and undifferentiated rectal cancer were significantly higher than those in patients with moderately differentiated and highly differentiated rectal cancer. It is suggested that CEA, CA199, and Hsp90α are highly expressed in serum of rectal cancer patients, and these are closely related to patients' Dukes stage and differentiation degree, which may provide a strong test to support early diagnosis of rectal cancer, which is similar to the results of Zou ChaoShi et al.[15].

In this study, univariate and multivariate Cox regression analysis was used to determine the relationship between CEA, CA199, and Hsp90α levels and the progression of rectal cancer ($P<0.05$). ROC curve analysis showed that the AUC of CEA in the diagnosis of rectal cancer was 0.714.AUC of CA199 in the diagnosis of rectal cancer was 0.768%, and the AUC of Hsp90α in the diagnosis of rectal cancer was 0.809. The AUC of combined diagnosis of colorectal cancer was 0.876. It is suggested that CEA, CA199, and Hsp90α have better predictive value and combined detection is more ideal than single detection in the diagnosis of rectal cancer. It is helpful for doctors to diagnose rectal cancer early and take appropriate action, which is of great significance for effective treatment of patients.

In conclusion, CEA, CA199, and Hsp90α levels are highly expressed in serum of patients with rectal cancer, and are closely related to patients' Dukes stage and differentiation. They are influencing factors for the progression of rectal cancer and play a certain role in the diagnosis of rectal cancer. The value of the three levels is highest when combined, a finding that can be widely used in clinical practice.

References

1) Turati L, Steccanella F, Petrelli F, Vitali E, Barni S, et al. Signet-ring cell carcinoma of the rectum and synchronous renal cell carcinoma in a young man. Int J Colorectal Dis 2016; 31(5): 1071-1072.
2) Pai A, Eftaiha SM, Melich G, Park JJ, Lin PK, et al. Robotic Site Adjusted Levator Transection for Carcinoma of the Rectum: A Modification of the Existing Cylindrical Abdominoperineal Resection for Eccentrically Located Tumors. World J Surg 2017; 41(2): 590-595.
3) Dutta SW, Alonso CE, Waddle MR, Khandelwal SR, Janowski EM, et al. Squamous cell carcinoma of the rectum: Practice trends and patient survival. Cancer Med 2018; 7(12): 6093-6103.
4) Kim CG, Ahn JB, Jung M, Beom SH, Heo SJ, et al. Preoperative Serum Carcinoembryonic Antigen Level as a Prognostic Factor for Recurrence and Survival After Curative Resection Followed by Adjuvant Chemotherapy in Stage III Colon Cancer. Ann Surg Oncol 2017; 24(1): 227-235.
5) Jia Y, Cavileer TD, Nagler JJ. Acute hyperthermic responses of heat shock protein and estrogen receptor mRNAs in rainbow trout hepatocytes. Comp Biochem Physiol A Mol Integr Physiol 2016; 201: 156-161.
6)
7) García R, Merino D, Gómez JM, Nistal JF, Hurlé MA, et al. Extracellular heat shock protein 90 binding to TGFβ receptor I participates in TGFβ-mediated collagen production in myocardial fibroblasts. Cell Signal 2016; 28(10): 1563-79.
8) Tu Y, Tian Y, Wu Y, Cui S. Clinical significance of heat shock proteins in gastric cancer following hyperthermia stress: Indications for hyperthermic intraperitoneal chemoperfusion therapy. Oncol Lett 2018; 15(6): 9385-9391.
9) Yang W, Luo Y, Hu S, Li Y, Liu Q. Value of combined detection of serum carcino-embryonic antigen, carbohydrate antigen 19-9 and cyclooxygenase-2 in the diagnosis of colorectal cancer. Oncol Lett 2018; 16(2): 1551-1556.
10) Wu D, Cao G, Huang Z, Jin K, Hu H, et al. Decreased miR-503 expression in gastric cancer is inversely correlated with serum carcinoembryonic antigen and acts as a potential prognostic and diagnostic biomarker. Onco Targets Ther 2016; 10: 129-135.
11) Shao W. Combined Determination of Carbohydrate Antigen 199 and SirT1 Based on an Electrochemiluminescence Immunosensor. Int J Electrochem Sci 2017; 11506-11514.
12) Chen X, Cai S, Li B, Zhang X, Li W, et al. Identification of key genes and pathways for esophageal squamous cell carcinoma by bioinformatics analysis. Exp Ther Med 2018; 16(2): 1121-1130.
13) Ju YT, Kwag SJ, Park HJ, Jung EJ, Jeong CY, et al. Decreased expression of heat shock protein 20 in colorectal cancer and its implication in tumorigenesis. J Cell Biochem 2015; 116(2): 277-86.
14) Valencia J, Gómez G, López W, Mesa H, Henao FJ. Relationship between HSP90a, NPC2 and L-PGDS proteins to boar semen freezability. J Anim Sci Biotechnol 2017; 8: 21.
15) Nomura M, Takahashi H, Fujii M, Miyoshi N, Haraguchi N, et al. Clinical significance of invasion distance relative to prognosis in pathological T3 colorectal cancer. Oncol Lett 2019; 18(5): 5614-5620.

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