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Original Article

Serum HE4, MASP-2, and DKK-1 levels in patients with colorectal cancer

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Abstract: Objective: To investigate the diagnostic performance of serum human epididymis protein 4 (HE4), mannose-binding lectin-associated serine protease 2 (MASP-2) and Dickkopf-1 (DKK-1) for colorectal cancer, and their correlations with patients' clinicopathological characteristics. Methods: 69 patients with colorectal cancer were enrolled in the study group (SG), and 60 healthy people receiving a physical examination at the same time were enrolled in the control group (CG). Enzyme-linked immunosorbent assays (ELISA) were used to measure the serum levels of HE4, MASP-2, and DKK-1 in the control and colorectal cancer groups at the time of pre-chemotherapy (T0), after the 3rd cycle of chemotherapy (T1), after the 6th cycle of chemotherapy (T2), and after the 8th cycle of chemotherapy (T3). The diagnostic ability of the combined measurement of HE4, MASP-2, and DKK-1 for colorectal cancer, and their correlations with the clinical pathology of colorectal cancer, were observed. Results: At T0, T1 and T3, the SG had statistically higher HE4 and DKK-1 levels and a lower MASP-2 level than the CG (all P<0.001). In the SG, the HE4 and DKK-1 levels were the highest at T0 and the lowest at T3 (P<0.001), but the MASP-1 level was the lowest at T0 and the highest at T3 (P<0.001). According to a Spearman correlation analysis, the HE4, DKK-1, and MASP-2 levels correlated with chemotherapy time (P<0.001). An ROC curve demonstrated that combined HE4, MASP-2, and DKK-1 have a diagnostic ability in colorectal cancer (P<0.001). The HE4 levels were statistically different in the different clinical stages, differentiation degrees, T stage, and N stage (P<0.001). The MASP-2 levels were statistically different (P<0.001) in the various differentiation degrees. The DKK-1 levels were statistically different in the different histological classifications, clinical stages, differentiation degrees, T stage, and N stage (P<0.001). Conclusion: The levels of HE4 and DKK-1 in the serum of patients with colorectal cancer are higher than in normal people, but the level of MASP-2 is lower, both of which are closely related to the clinicopathology of colorectal cancer. The combined measurement has a good diagnostic functional ability for colorectal cancer and is expected to become an excellent indicator for the diagnosis and treatment of colorectal cancer.

Keywords: HE4, MASP-2, DDK-1, colorectal cancer (CC)

Introduction

Colorectal cancer (CC) is the most common malignant tumor of the gastrointestinal tract, usually originating in the large intestinal mucosa [1]. At present, the incidence of CC is third among all malignant tumors and second only to gastric and esophageal cancer [2]. CC patients are usually middle-aged, but in recent years there is a significant trend of the cancer occurring in younger people [5]. There are no specific features for patients with early CC, and it is more likely that patients will reach the middle and later stages after their diagnosis, and the treatment difficulty is further ameliorated [6]. At present, the treatment of CC in the clinic mainly involves chemoradiotherapy or surgery, but the prognosis is not satisfactory. It is reported that the one-year mortality of advanced colorectal cancer patients is as high as 9.20% [7]. Therefore, researchers at home and abroad are constantly striving to find new directions for CC early diagnosis and treatment.

HE4 is an acidic protein that is mainly expressed in the epithelial cells of the distal epithelial cells of the epididymis and the vas deferens. At present, HE4 is involved in the occurrence and development of malignant tumors such as ovarian cancer, endometrial cancer, and lung cancer [8-10]. Nagy et al. [11] suggested that HE4 is a novel serum inflammatory biomarker in cys-

tic fibrosis. MASP-2 is a key enzyme of the complement lectin pathway closely related to chronic infection and autoimmune diseases. Hertle et al. [12] showed that MASP-2 is closely related to endothelial dysfunction and the thickness of the intima-media layer. Dickkopf-1 (DKK-1) is a well-established inhibitor of the Wnt/β-catenin signaling pathway and can effectively influence the biological behavior of tumors [13]. The pathogenesis of CC is closely related to the Wnt/β-catenin signaling pathway [14], so it is thought that DKK-1 also has a significant effect on CC. At present, there were still few studies on HE4, MASP-2, and DKK-1 related CCs. Whether HE4, MASP-2, and DKK-1 have a relationship with CC has not been determined yet. In this study, we analyzed the levels of HE4, MASP-2, and DKK-1 in CC patients and their clinical significance for CC.

Materials and methods

General information

69 patients with CC admitted to our hospital and 60 healthy people receiving a physical examination at the same time were enrolled. The patients with CC were placed in the study group (SG, n=69), and the healthy patients were placed in the con (CG, n=60). The patients in the SG were 42 to 69 years old, with an average age of (53.64±10.77) years, and there were 21 females and 48 males. The patients in the CG were 40-70 years old, with an average age of (54.08±10.24) years, and there were 42 males and 18 females. This experiment was approved by the Ethics Committee of Qilu Hospital of Shandong University, and all the study participants signed an informed consent.

Inclusion and exclusion criteria

Inclusion criteria for SG: the patients should be in line with the CC clinical manifestations [15]; patients who were diagnosed with CC by pathology biopsy in our hospital; patients who received follow-up treatment in our hospital after their diagnoses; patients with complete case reports; patients who cooperated with the medical personnel; people who underwent no adjuvant therapy before the study. Inclusion criteria for CG: people without any disease; people with normal physical examination results; people with an age range from 40 to 70 years; people who cooperated with this study.

Exclusion criteria for SG: patients who also had other tumors; patients who also had other cardiovascular and cerebrovascular diseases; patients who also had organ failure; patients with hepatorenal insufficiency; patients with mental illness; patients with a physical handicap; patients who could not take care of themselves during long periods of being bedridden; patients who were had by autoimmune diseases; patients who were transferred to another hospital; patients with surgical contraindications.

Methodology

Patients in the SG underwent a radical resection of CC after admission. The operation was performed by senior clinical surgeons in our hospital. One month after surgery, a Xeloda (capecitabine) and oxaliplatin (XELOX) regimen was applied for the patients in the SG. Oxaliplatin was given by intravenous infusion to the patients at 130 mg/m^2 on the first day of the treatment cycle, and capecitabine was given orally at 1000 mg/m^2 on the 1st-14th days, twice a day. After 14 days of continuous administration, the drug was stopped for 7 days, and then every 3 weeks was a treatment cycle, for a total of 8 cycles. In the CG and the SG, 5 mL of fasting venous blood was taken before treatment (T0), 3 cycles after treatment (T1), 6 cycles after treatment (T2), and 8 cycles after treatment (T3). The blood was placed at room temperature for 30 min and centrifuged for 10 min (4000 rpm/min), then the supernatant was obtained. The serum levels of HE4, MASP-2, and DKK-1 were measured using an ELISA. The HE4 kits were purchased from Shanghai Qunji Biotechnology Co., Ltd. (batch number KA-4025). The MASP-2 kits were purchased from Wuhan Boouter Biotech Co., Ltd. (batch number orb440678). The DKK-1 kits were purchased from Beijing Luyuan Bird Biotechnology Co., Ltd. (batch number SEK10170).

Outcome measures

The serum levels of HE4, MASP-2, and DKK-1 were measured in the two groups. The diagnostic value of the combined measurement of HE4, MASP-2, and DKK-1 at T1 for CC was used. SPSS was used to do a logistic binary regression analysis to determine the joint predictors

Table 1. Comparison of the clinical data [n (%)]

	Study group (n=69)	control group (n=60)	t or χ^2	*P*
Age	53.64±10.77	54.08±10.24	0.237	0.813
BMI (cm/kg^2)	25.13±6.72	25.63±6.28	0.435	0.665
WBC (× 10^9/L)	6.24±2.57	6.64±1.90	0.992	0.323
RBC (× 10^{12}/L)	4.14±1.07	4.29±1.24	0.738	0.462
PLT (× 10^9/L)	256.13±46.97	249.18±40.16	0.896	0.372
Gender			0.003	0.957
Male	48 (69.57)	42 (70.00)		
Female	21 (30.43)	18 (30.00)		
Living Environment			0.688	0407
Cities and towns	59 (85.51)	48 (80.00)		
Rural	10 (14.49)	12 (20.00)		
Smoking			0.616	0.433
Yes	46 (66.67)	36 (60.0)		
no	23 (33.33)	24 (40.00)		
Drinking			0.454	0.500
Yes	42 (60.87)	33 (55.00)		
no	27 (39.13)	27 (45.00)		
Sport			0.714	0.398
Yes	6 (8.70)	8 (13.33)		
no	63 (91.30)	52 (86.67)		
Nationality			0.000	0.978
Han nationality	62 (89.86)	54 (90.00)		
minority	7 (10.14)	6 (10.00)		
Marital status			0876	0.349
married	68 (98.55)	60 (100.00)		
unmarried	1 (1.45)	0 (0.00)		
Tumor classification				
Polypoid	19 (27.54)			
Flat bulge	16 (23.19)			
Flat hump with ulceration	14 (20.29)			
Lump type	8 (11.59)			
Ulcer type	6 (8.70)			
Infiltrating type	6 (8.70)			
Organizational classification				
adenocarcinoma	49 (71.01)			
mucinous carcinoma	13 (18.84)			
undifferentiated carcinoma	7 (10.14)			
Clinical stage				
I-II	36 (52.17)			
III-IV	33 (47.83)			
Differentiation				
Undifferentiated	21 (30.43)			
Medium and high differentiation	48 (69.57)			
T staging				
T1 + T2	32 (46.38)			
T3 + T4	37 (53.62)			
N staging				

N0	20 (28.99)			
N1 + N2 + N3	49 (71.01)			
Family history of diseases			68.415	<0.001
Yes	52 (75.36)	2 (3.33)		
No	17 (24.64)	58 (96.67)		
Food preference			42.092	<0.001
Vegetarianism	7 (10.14)	39 (65.00)		
Carnivorism	62 (89.86)	21 (35.00)		
History of intestinal inflammation			25.853	<0.001
Yes	39 (56.52)	8 (13.33)		
No	30 (43.48)	52 (86.67)		

of the three indicators. An ROC curve was used to analyze the diagnostic value of HE4, MASP-2 and DKK-1 for the combined detection of CC. Changes in the course of treatment of HE4, MASP-2, and DKK-1, and the correlations between HE4, MASP-2, and DKK-1 and the clinical pathology were used.

Statistical methods

All the experimental results were calculated using SPSS 24.0 statistical software (Shanghai Yuchuang Network Technology Co., Ltd.). All the graphics were drawn using GraphPad 8 (Shenzhen Tianrui Software Technology Co., Ltd.) software, and the results were checked twice. The count data were expressed in terms of the rate, including patient gender, living environment, etc. Chi-squared tests were used for the comparisons between groups. The measurement data such as HE4 and MASP-2 concentrations were expressed in the form of mean ± standard deviation. Multiple time-points data were compared using the repeated measures ANOVA and Bonferroni post-mortem. An independent *t* test was used for the comparisons between groups. The diagnostic values were analyzed using ROC curves. A logistic regression model was established to explore the significance of the cut-off for the joint diagnosis, and a Spearman correlation was used for the correlation analysis. P<0.050 was considered statistically significant.

Results

Comparison of the clinical data

Age, BMI, white blood cells (WBC), red blood cells (RBC), platelets (PLT), gender, living environment, smoking, research, exercise status, ethnicity, and marital status were compared between the two groups, and there were no significant differences (P>0.050), which proved that the two groups of patients were comparable. Compared with the CG, the subjects in the SG had a family history of diseases, a preference for carnivorism, and a history of intestinal inflammation (P<0.05) (**Table 1**).

The serum levels of HE4, MASP-2, and DKK-1 in the two groups

The serum HE4 concentrations of the SG at T0, T1, T2, and T3 were (31.84±9.07) μg/L, (27.76±7.39) μg/L, (24.24±4.53) μg/L, and (22.05±5.12) μg/L, respectively. The serum HE4 concentration of the CG was (21.54±4.62) μg/L.

The serum MASP-2 concentrations of the SG at T0, T1, T2, and T3 were (312.77±116.24) ng/mL, (379.54±105.93) ng/mL, (422.06±86.77) ng/mL, and (469.83±134.96) ng/mL, respectively. The serum MASP-1 concentration of the CG was (475.53±184.09) ng/mL.

The serum DKK-1 concentrations of the SG at T0, T1, T2, and T3 were (42.33±12.68) pg/mL, (33.85±15.07) pg/mL, (24.70±8.52) pg/mL, and (19.04±5.12) pg/mL, respectively. The serum DKK-1 concentration of the CG was (18.62±4.87) pg/mL.

In the SG, serum HE4, MASP-2, and DKK-1 concentrations were not different from those in the CG at T3 (P>0.050), and the serum HE4 and DKK-1 levels were higher than those in the CG at T0, T1, and T3 (P<0.001), and the MASP-2 level was lower than it was in the CG (P<0.001).

In the SG, HE4, and DKK-1 were the highest at T0 (P<0.001), and those were lower at T2 than

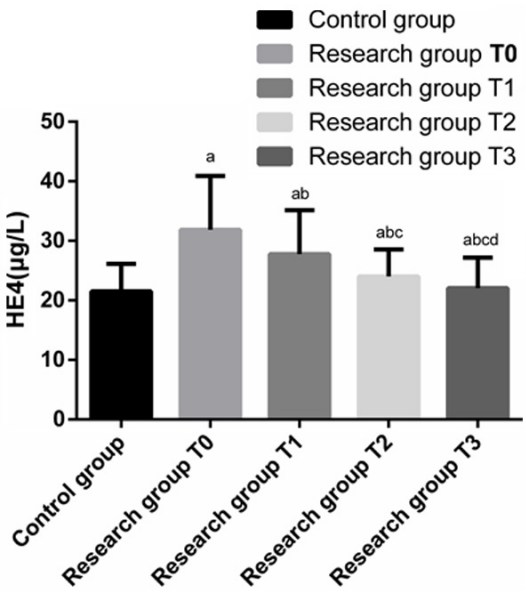

Figure 1. Comparison of the serum HE4 concentrations between the two groups. a indicated P<0.001 compared to the serum HE4 concentration in the control group. b indicated P<0.001 compared with the serum HE4 concentration in the study group at T0. c indicated P<0.001 compared with the serum HE4 concentration of the study group at T1. d indicated P<0.001 compared with the serum HE4 concentration of the study group at T2.

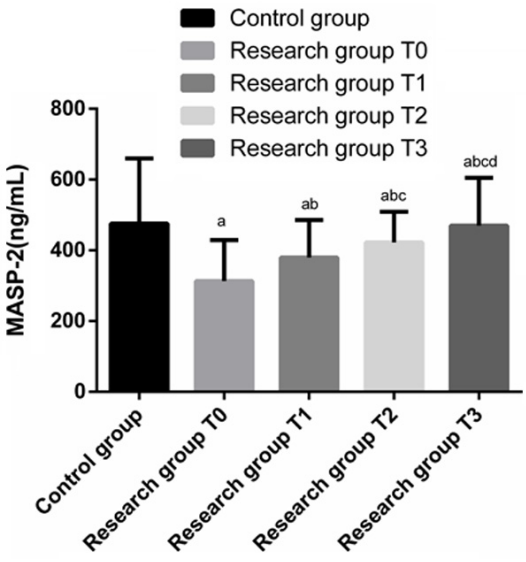

Figure 2. Comparison of the serum MASP-2 concentrations between the two groups. a indicated P<0.001 compared to the serum MASP-2 concentration in the control group. b indicated P<0.001 compared with the serum MASP-2 concentration of the study group at T0. c indicated P<0.001 compared with the serum MASP-2 concentration of the study group at T1. d indicated P<0.001 compared with the serum MASP-2 concentration of the study group at T2.

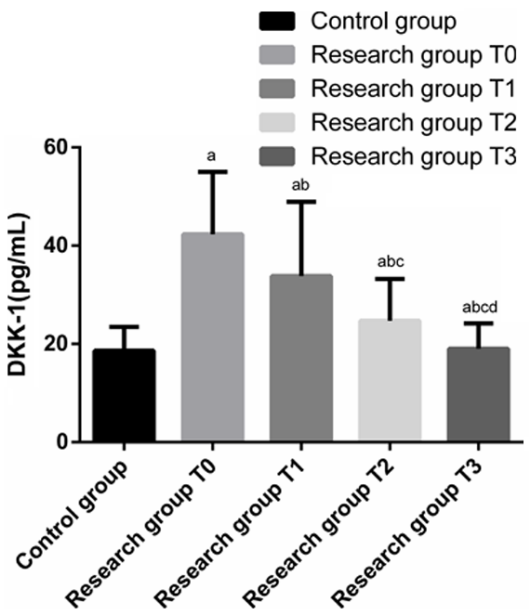

Figure 3. Comparison of the serum DKK-1 concentrations between the two groups. a indicated P<0.001 compared to the serum DKK-1 concentration in the control group. b indicated P<0.001 compared with the serum DKK-1 concentration of the study group at T0. c indicated P<0.001 compared with the serum DKK-1 concentration of the study group at T1. d indicated P<0.001 compared with the serum DKK-1 concentration of the study group at T2.

T1, and lower at T3 than T2 (P<0.001). MASP-1 was the lowest at T0 (P<0.001), which was higher at T2 than T1, and higher at T3 than T2 (P<0.001) (**Figures 1**-**3**).

Changes in HE4, MASP-2, and DKK-1 during treatment

A Spearman correlation analysis showed that HE4 was negatively correlated with treatment (r=-0.503, P<0.001). MASP-2 had a positive correlation with treatment (r=0.325, P<0.001). There was a negative correlation between DKK-1 and treatment (r=-0.642, P<0.001) (**Table 2**; **Figures 4**-**6**).

The diagnostic efficacy of HE4, MASP-2, and DKK-1 for CC

According to the ROC curve analysis, when the cut-off value was 25.91 μg/L, the sensitivity of the HE4 single-term diagnosis of CC was 69.57%, and the specificity was 84.06%. When it was 518.90 ng/mL, the sensitivity of the MASP-2 single-term diagnosis of CC was 98.55%, and the specificity was 52.17%. When

Table 2. Correlation between the HE4, MASP-2, and DKK-1 levels and the time of chemotherapy

	HE4	MASP-2	DKK-1
r	-0.503	0.325	-0.642
95% CI	-0.589~-0.467	0.212~0.430	-0.708~0.564
P	<0.001	<0.001	<0.001

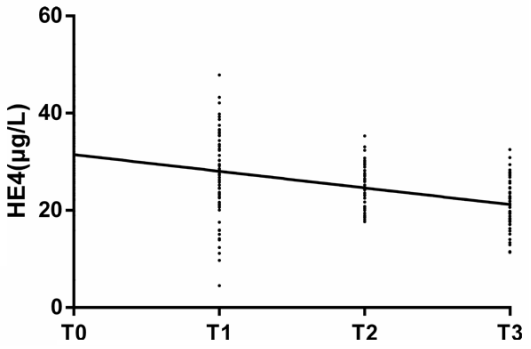

Figure 4. A correlation analysis of HE4 and treatment time in the study group. According to the Spearman correlation analysis, HE4 was negatively correlated with treatment (r=-0.503, P<0.001).

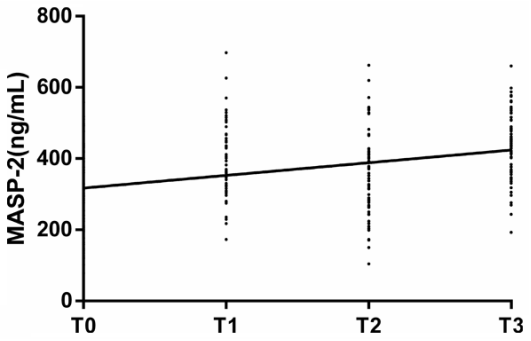

Figure 5. A correlation analysis of MASP-2 and treatment time in the study group. According to the Spearman correlation analysis, MASP-2 was positively correlated with treatment (r=0.325, P<0.001).

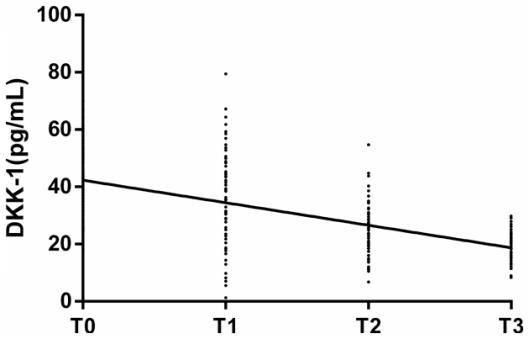

Figure 6. Correlation analysis of DKK-1 and treatment time in the study group. According to the Spearman correlation analysis, DKK-1 was negatively correlated with treatment (r=-0.642, P<0.001).

it was 24.01 pg/mL, the sensitivity of the DKK-1 single-diagnosis of CC was 78.26% and the specificity was 91.30%. Using HE4, MASP-2, and DKK-1 as independent variables for the logistic binary regression analysis, the regression model Logit(P)=-4.285 + 0.017 × HE4 + (-0.003) × MASP-2 + 0.211 × DKK-1 was obtained. When the cut-off value was 0.52, the sensitivity of the model for the diagnosis of colorectal cancer was 86.96%, and the specificity was 89.86% (**Table 3**; **Figures 7**-**10**).

The correlation between HE4, MASP-2, DKK-1 and clinical pathology

There was no difference in the expression levels of HE4 in the different tumor and tissue types (P>0.050). There were differences in the HE4 levels in the different clinical stages, differentiation, T stage and N stage (P<0.001). There was no difference in the expression levels of MASP-2 in the different tumor types, tissue types, clinical stages, T stages, and N stages (P>0.050), but there were differences in the expression levels in the various degrees of differentiation (P<0.001). The DKK-1 expression levels were not different in the different tumor types (P>0.050), but there were differences in the different tissue types, clinical stages, differentiation degrees, T stage and N stage (P<0.001) (**Table 4**).

Discussion

At present, the incidence and mortality of CC are increasing day by day, making CC a hot spot of clinical research [16]. With the intensification of this research, more and more serum proteins, genes, and cytokines have been shown to be closely related to CC. Therefore, it is extremely important to find an accurate indicator of CC occurrence, development, and change, and a convenient means of detection. At present, the relationships between HE4, MASP-2, and DKK-1 and CC are not clear, but HE4, MASP-2, and DKK-1 are all serum factors, and the measurement method is convenient. And studies have shown that HE4, MASP-2, and DKK-1 have strong specificities [17-19]. Therefore, the determination of the expression levels of HE4, MASP-2, and DKK-1 in CC patients is of great significance not only for the early screening of CC, but also for the observation of the recovery of tumors in subsequent treatment.

The results of this experiment showed that the serum levels of HE4 and DKK-1 in CC patients

Table 3. Diagnostic efficacy of HE4, MASP-2, and DKK-1 for CC

	HE4	MASP-2	DKK-1	Joint diagnosis
Area	0.810	0.738	0.876	0.939
Std. error	0.037	0.045	0.031	0.020
95% CI	0.736~0.883	0.650~0.827	0.815~0.936	0.899~0.978
P	<0.001	<0.001	<0.001	<0.001
Cut-off	25.91 μg/L	518.90 ng/mL	24.01 pg/mL	0.52
Sensitivity (%)	69.57	98.55	78.26	86.96$^{*,\#,\Delta}$
Specificity (%)	84.06	52.17	91.30	89.86$^{\#}$

Note: *indicates P<0.050 when the sensitivity and specificity of joint diagnosis were compared with those of HE4 single diagnosis. $^{\#}$indicates P<0.050 when the sensitivity and specificity of joint diagnosis were compared with those of the MASP-2 single diagnosis. $^{\Delta}$indicates P<0.050 when the sensitivity and specificity of the joint diagnosis were compared with those of DKK-1 single diagnosis.

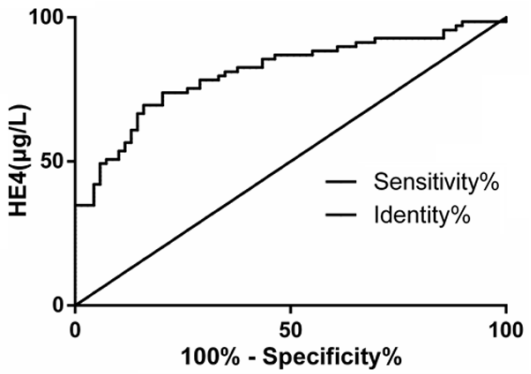

Figure 7. Analysis of the effect of HE4 single detection for the diagnosis of CC. According to the ROC curve analysis, when the cut-off value was 25.91, the sensitivity of the HE4 single diagnosis CC is 69.57%, and the specificity was 84.06%.

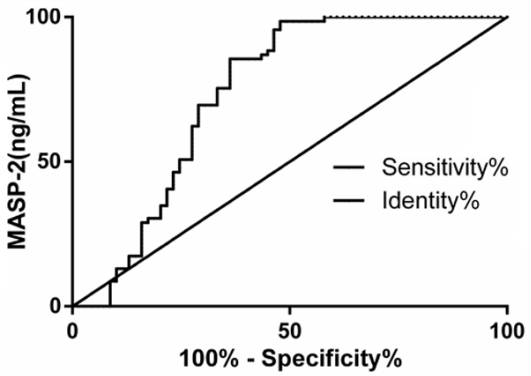

Figure 8. Analysis of the effect of the MASP-2 single diagnosis of CC. According to the ROC curve analysis, when the cut-off value was 518.90, the sensitivity of the MASP-2 single diagnosis CC was 98.55%, and the specificity was 52.17%.

are significantly higher than those in the normal population, and their MASP-2 levels are significantly lower than they are in the normal population, levels which gradually change with the treatment process. This suggests that HE4, MASP-2, and DKK-1 may be involved with the occurrence and development of CC. This conclusion is consistent with the findings of Kemal et al. [20], Maestri [21], Qiao [22], etc. HE4 is a whey acidic protein mainly distributed in the epithelium of the reproductive system and has been shown to be closely related to innate immunity [23]. HE4 was first used to diagnose tumors in the reproductive system. However, recent research shows that HE4 is not only closely related to reproductive system tumors, but it's also abnormally expressed in malignant tumors such as gastric cancer and pancreatic adenocarcinoma [24, 25]. The serum HE4 concentrations of the CC patients in this study were significantly higher than those of the normal population, and there were differences in the different clinical stages, differentiation degree, T stage, and N stage of CC. This suggests that HE4 gradually increases with the development of tumors in CC and can be used as an excellent indicator for monitoring the development of CC in the future. We speculate that the reason for the abnormal level of HE4 in CC was that HE4 has the characteristics of a trypsin inhibitor [26]. Trypsin can be reduced to aprotinin in humans, relying on tissue epithelial cells to maintain its cell function integrity [27]. The body tissues of patients with CC have been damaged by the attacks of the tumor cells. At this time, HE4 further inhibits the protective effect of trypsin on cell function, which makes the spread and metastasis of tumor cells more rapid, thereby aggravating a patient's condition.

In this study, MASP-2 only had differences in the expressions of CC in the various degrees of differentiation, and it had no significant relationship with any other pathological features. The reasons for this are presumed to be as follows: 1. Because the number of cases in this experimental study was too small, the test results were accidental. 2. MASP-2 is a serum factor that is highly specific for tumor detection.

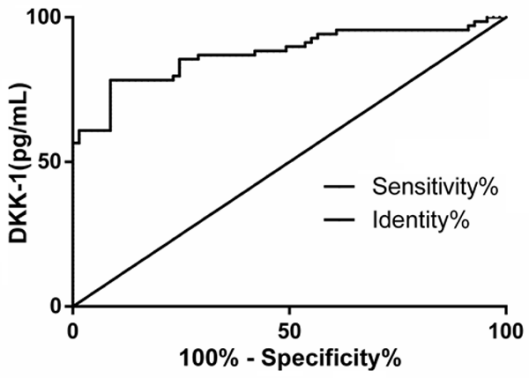

Figure 9. Analysis of the effect of DKK-1 single diagnosis CC. According to the ROC curve analysis, when the cut-off value was 24.01, the sensitivity of the DKK-1 single diagnosis CC was 78.26%, and the specificity was 91.30%.

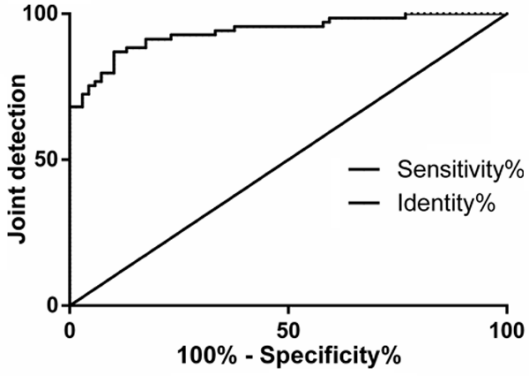

Figure 10. Analysis of the CC effects of HE4, MASP-2, and DKK combined detection. According to the ROC curve analysis, when the cut-off value was 0.52, the sensitivity of the HE4, MASP-2 and DKK-1 combined diagnosis of CC was 86.96%, and the specificity was 89.86%.

MASP-2 may be involved in the development and progression of tumors through complement-activated lectins [28]. The degree of differentiation represents the similarity between tumor cells and normal cells. The lower the degree of differentiation, the higher the similarity between tumor cells and normal cells. MSAP-2 does not recognize the difference between the two, so it cannot play a role in cell adhesion. The higher the differentiation degree of tumor cells, the greater their difference from normal cells. MASP-2 acts as a lectin to link different cells together. However, SWIERZKO et al. [29] found that the level of MASP-2 is significantly higher than it is in healthy people when diagnosing ovarian cancer patients, which is different from this experiment. This may be due to different tumors.

DDK-1 has been shown to inhibit the Wnt/β-catenin signaling pathway and regulate cell differentiation and development, resulting in inducing tumor cell proliferation and migration [30]. In this experiment, DKK-1 was associated with CC tissue classification, clinical stage, differentiation, T stage, and N stage. We speculated that the tumor proliferation may activate the inhibitory effects of DKK-1 on the Wnt channels, showing a high expression state to inhibit tumor proliferation and exert negative feedback regulation.

The combined measurement of HE4, MASP-2 and DKK-1 serves as a good diagnostic tool for CC, suggesting that it can be used as a screening indicator for CC in the future. This is also consistent with the conclusions put forth by Dayyani et al. [31], that is, the measurement of HE4 combined with other cancer markers is of higher value for tumor diagnosis.

Since there are few related studies of HE4, MASP-2, and DKK-1 in CC, it is impossible to refer to more experimental results for analysis and comparison. Due to our limited experimental conditions, the mechanism of action of HE4, MASP-2, and DKK-1 in CC has not been experimentally verified. More in-depth experimental analysis will be carried out as soon as possible to confirm the relevant mechanisms of HE4, MASP-2, and DKK-1, and the size of the study cohort will be expanded to obtain more reliable data results. In addition, the subjects of this experiment will be followed up for a longer period of time to provide a longer-term data analysis for the clinic.

In summary, the concentrations of HE4, MASP-2, and DKK-1 in CC patients are significantly higher than they are in the normal population, and they change with the course of treatment. The combined measurement of HE4, MASP-2, and DKK-1 serves as a good diagnostic tool for CC.

Disclosure of conflict of interest

None.

Table 4. The correlation between HE4, MASP-2, and DKK-1 and clinical pathology

	n	HE4	MASP-2	DKK-1
Tumor classification				
Polypoid	19	32.37±8.11	324.15±121.52	42.18±11.98
Flat bulge	16	33.04±8.56	332.24±117.74	42.51±12.15
Flat hump with ulceration	14	32.16±8.16	328.19±124.51	42.89±11.57
Lump type	8	32.75±9.04	324.72±118.51	43.08±11.69
Ulcer type	6	33.10±8.86	330.74±120.15	42.47±12.56
Infiltrating type	6	32.54±8.75	325.75±129.47	43.10±12.83
Organizational classification				
adenocarcinoma	49	33.42±9.24	319.44±115.15	48.56±12.51
mucinous carcinoma	13	32.87±8.92	325.42±121.57	42.15±11.71*
undifferentiated carcinoma	7	33.04±8.77	320.74±120.94	43.23±10.86
Clinical stage				
I-II	36	35.15±10.27	325.44±124.05	40.56±9.84
III~IV	33	29.54±7.14*	314.45±135.48	47.51±8.62*
Differentiation				
Low differentiation	21	34.21±9.54	264.42±140.62	48.55±10.51
Medium and high differentiation	48	28.15±6.33*	354.72±118.72*	42.63±7.06*
T staging				
T1 + T2	32	28.89±6.54	316.11±125.41	41.16±7.26
T3 + T4	37	34.82±10.51*	327.41±115.70	49.72±10.01*
N staging				
N0	20	26.51±6.11	320.42±125.15	39.54±8.42
N1 + N2 + N3	49	33.11±8.42*	316.44±118.62	47.83±9.09*

Note: *indicated a statistical difference.

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