Serum Dihydropyrimidinase-Like 3 Concentration in Patients with Gastric Cancer and Its Diagnostic Value

*Huiqiu Zhong [1], *Xiaojiang Luo [2]*

1. *Department of Otorhinolaryngology Head and Neck Surgery, Jiangxi Provincial People's Hospital, Nanchang 330006, P.R. China*
2. *Department of Gastrointestinal Surgery, Jiangxi Provincial People's Hospital, Nanchang 330006, P.R. China*

Corresponding Author: Email: jbm2k8@163.com

Abstract

Background: We aimed to investigate the serum concentration of dihydropyrimidinase-like 3 (DPYSL3) in patients with gastric cancer and its clinical significance.

Methods: Seventy four patients with gastric cancer from Wuhan Central Hospital, Tongji Medical College, Huazhong University of Science and Technology, China from October 2018 to April 2019 were selected as the case group. Sixty patients with normal gastric mucosa or mild non-atrophic gastritis were selected as the control group. Serum DPYSL3, CA72-4 and CEA concentrations were measured in both groups.

Results: The serum DPYSL3 concentration in the case group was significantly higher than that in the healthy control group (22.04 ± 9.22 vs. 8.36 ± 4.19 µg/L, $P<0.001$). The serum DPYSL3 concentration in patients with advanced gastric cancer was significantly higher than that in early gastric cancer (27.09 ± 9.12 vs. 13.04 ± 8.22 µg/L, $P<0.01$); serum DPYSL3 concentration was significantly correlated with tumor size, TNM stage and differentiation ($P<0.05$). When the cutoff value was 20.98 µg/L, the serum DPYSL3 concentration could differentiate the gastric cancer with ROC^{AUC} 0.882 (95% CI: 0.828-0.937) with sensitivity and specificity of 75% and 94%, respectively. Serum CA72-4 concentration could differentiate the gastric cancer from health controls with ROC^{AUC} 0.812 (95% CI: 0.734-0.834), serum CEA concentration could differentiate gastric cancer with ROC^{AUC} 0.612 (95% CI: 0.534 ~ 0.634). The serum concentrations of DPYSL3, CA72-4 and CEA in gastric cancer patients were increased compared to health controls.

Conclusion: Three serological markers have complementary diagnostic value for gastric cancer. Serum DPYSL3 is a new potential molecular marker for gastric cancer.

Keywords: Serum; Carcinoembryonic antigen; Gastric cancer; Diagnosis; Dihydropyrimidinase-like 3 protein

Introduction

Gastric cancer is a common malignant tumor on a global scale and one of the malignant tumors with high mortality. China is a high-risk area of gastric cancer. The number of cases and the number of deaths each year account for about 45% of the world's total, with more than 1 million new cases each year and about 350,000 deaths (1).

The prognosis of patients with gastric cancer is closely related to the diagnosis and treatment period. Early gastric cancer can be completely cured by endoscopic or surgical treatment. The 5-year

survival rate can exceed 90%, while the 5-year survival rate of advanced gastric cancer is less than 30% (2). Early diagnosis and screening are of great significance for improving the prognosis of patients with gastric cancer. Early diagnosis of gastric cancer lacks non-invasive markers. Common serum markers such as carcinoembryonic antigen (CEA), CA199 and CA72-4 have low specificity and sensitivity, which is not conducive to early gastric cancer screening (2). Finding new serum markers for early diagnosis of gastric cancer has become a hot research topic.

Dihydropyrimidinase-like 3 (DPYSL3) is a cell adhesion molecule that is widely expressed in normal tissues such as the brain and pineal gland, and is moderately expressed in normal gastric mucosa (3). As a specific cell adhesion molecule, studies have shown that it plays an important role in tumorigenesis and progression, especially in tumor cell metastasis (4). Recently, the expression of DPYSL3 was significantly increased in cancer tissues of patients with stage IV gastric cancer, and the concentration of DPYSL3 protein in serum of patients with gastric cancer was significantly increased, which was an independent risk factor for poor prognosis of gastric cancer patients (5). Despite this, the concentration of Dihydropyrimidinase-like 3 protein in the serum of patients with gastric cancer and its clinical significance have not been reported at home and abroad.

This study was designed to examine the serum Dihydropyrimidinase-like 3 concentration in patients with gastric cancer and its clinical significance, and to compare its diagnostic value with conventional gastric cancer serum markers CA72-4 and CEA.

Materials and Methods

Study subjects

Seventy four patients with gastric cancer admitted to Wuhan Central Hospital affiliated to Tongji Medical College, Huazhong University of Science and Technology, China from October 2018 to April 2019 were enrolled. They included 46 males and 28 females, aged 61.09±11.45 years old.

Inclusion criteria were ① age 35-82 years; ② patients with primary gastric cancer who did not receive any radiotherapy or chemotherapy before surgery; ③ were diagnosed by pathological examination.

Exclusion criteria were ①Those who did not meet the above conditions; ② Patients with other tumors at the same time, ③ Patients with severe liver and kidney dysfunction; ④ Patients were unwilling to participate in the study.

At the same time, 60 healthy subjects in the hospital were collected as a control group, 30 males and 30 females, aged 58.4±7.5 years. The patients in the control group went through physical examinations, and their gastroscopy showed normal gastric mucosa or mild non-atrophic gastritis, there were no cancer in any other area.

The study was approved by the Hospital Medical Ethics Committee and all patients signed informed consent.

Methods

The fasting peripheral venous blood samples of the two groups were collected and centrifuged at room temperature for 10 min at 3000 r/min. The serum was stored in a -80 °C refrigerator and Dihydropyrimidinase-like 3 concentration in serum samples was tested by ELISA. The procedure followed the instructions provided by the human DPYSL3 ELISA kit (Boster biological technology, Pleasanton, CA). The traditional tumor markers CA72-4 and CEA were detected by electrochemiluminescence immunoassay. CA72-4≥6.95 IU/mL and CEA≥15ng/mL showed that the test results were positive.

Statistical methods

All data were analyzed by SPSS19.0 statistical software package (Chicago, IL, USA) and statistical analysis was performed. The measurement data were expressed in the form of mean ± standard deviation. The two groups were compared by using independent sample t test. One-way analysis of variance was used for comparison among groups. The least significant difference

method (LSD method) was used for multiple comparison. *P<0.05* for the difference was statistically significant.

Results

Comparison of serum DPYSL3, CA72-4 and CEA concentrations in the two groups

The serum DPYSL3 concentration in the case group was significantly higher than that in the healthy control group (22.04±9.22 vs. 8.36±4.19 µg/L, *P<0.001*). The concentrations of CA72-4 and CEA in the case group were significantly higher than those of healthy controls (*P<0.05*) (Table 1).

Table 1: Comparison of serum DPYSL3, CEA and CA72-4 concentrations in the two groups

Group	Number of cases	DPYSL3 (µg/L)	CEA (ng/L)	CA72-4 (IU/mL)
Control	60	8.36±4.19	3.23±0.58	4.51±2.19
Case	74	22.04±9.22*	12.54±8.36*	52.78±11.74*
t		28.97	21.34	10.76
P		<0.001	<0.01	<0.001

*P<0.05 was considered significant

Comparison of serum DPYSL3, CA72-4 and CEA levels in patients with early gastric cancer and advanced gastric cancer

The serum DPYSL3 concentration in patients with advanced gastric cancer was significantly higher than that in early gastric cancer (27.09±9.12 vs. 13.04±8.22 µg/L, *P<0.01*). The serum levels of CA72-4 and CEA in patients with advanced gastric cancer were significantly higher than those in healthy controls (*P<0.05*), and significantly higher than those in early gastric cancer patients (*P<0.05*). There is no difference in serum CEA concentration between early gastric cancer patients and healthy controls (Table 2).

Table 2: Comparison of serum DPYSL3, CA72-4 and CEA levels in early gastric cancer and advanced gastric cancer

Group	Number of cases	DPYSL3 (µg/L)	CEA (ng/L)	CA72-4 (IU/mL)
Control	60	8.36±4.19	1.23±0.58	14.51±2.19
Early gastric cancer	32	13.04±8.22[a]	1.54±0.36	28.78±8.74[a]
Advanced gastric cancer	42	27.09±9.12[a,b,c]	12.54±8.36[b,c]	52.78±11.74[a,b,c]
F		28.97	21.34	10.76
P		<0.001	<0.01	<0.001

Note: a. Early gastric cancer compared with healthy control group, *P<0.05*; b advanced gastric cancer compared with healthy control group, *P<0.05*; c early gastric cancer compared with advanced gastric cancer, *P<0.05*. **P<0.05* was considered significant

Relationship between serum DPYSL3 concentration and clinical pathological parameters

Serum DPYSL3 concentration was not significantly correlated with gender, age and tumor location, serum DPYSL3 concentration was significantly correlated with tumor size, TNM stage and differentiation (*P < 0.05*) (Table 3).

Table 3: Relationship between serum DPYSL3 concentration and clinicopathological parameters in patients with gastric cancer

Clinical pathological parameters	Number of cases (n)	DPYSL3 concentration (ug/l)	t	P
Gender			1.45	0.459
Male	46	18.04±7.22		
Female	28	19.04±8.27		
Age			2.49	0.862
≤60 years	34	19.24±7.28		
>60 years	40	21.14±8.27		
Tumor size			12.48	0.003*
≤4 cm	41	19.24±7.22		
>4 cm	33	27.14±9.23		
TNM staging			12.26	0.002*
Stage I+II	34	16.74±7.13		
Stage III+IV	40	29.14±11.25		
Differentiation			11.09	0.001*
High-medium differentiation	44	16.74±7.13		
Low differentiation	30	29.14±11.25		
Tumor site			1.345	0.671
Gastric body	22	20.24±6.17		
Cardia	21	21.25±6.25		
Gastric antrum	21	20.18±5.91		

P<0.05 was considered significant

Diagnostic value of serum DPYSL3 concentration in gastric cancer

According to ROC curve analysis, when the cut-off value was 20.98 µg/L, the area under the curve of diagnosis of gastric cancer (AUC) was 0.882 (95% CI: 0.828-0.937), with 75% and 94% sensitivity and specificity, respectively. When the cutoff value was 5 ng/mL, the area under the curve of CEA for diagnosis of gastric cancer (AUC) was 0.612 (95% CI: 0.534 to 0.634), and the sensitivity and specificity was 39% and 86%, respectively. When the cutoff value was 18.34 IU/mL, the area under the curve (AUC) of CA72-4 diagnosed gastric cancer was 0.812 (95% CI: 0.734-0.834), and the sensitivity and specificity was 65% and 90%, respectively (Fig. 1). Serum DPYSL3 concentration was superior to traditional marker CA72-4 and non-specific serum tumor marker CEA in the diagnosis of gastric cancer.

Discussion

In recent years, with the continuous improvement of people's living standards, the incidence of gastric cancer has also increased year by year. The main risk factors for gastric cancer include stomach history, family history, smoking and drinking, and unhealthy food. The incidence of gastric cancer in China is second only to lung cancer, gastric cancer is becoming the second most common malignant tumor (6).
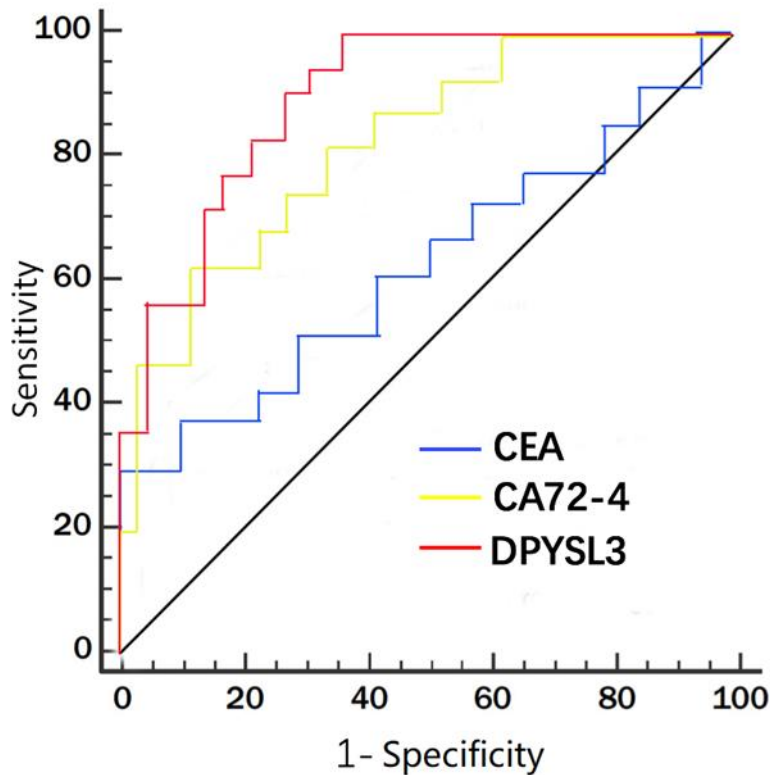
Fig. 1: The diagnosis ROC curve for three serum biomarkers in gastric cancer patients

The accepted standard for the diagnosis of gastric cancer is still gastroscopy biopsy. However, when tumor is detected by this method, most patients have already advanced into metastasis, and biopsy has the disadvantages of high cost, patient suffering, and high technical requirements (7). Patients with gastric cancer usually have no special symptoms at the beginning of the disease, and early diagnosis is difficult. Many patients with gastric cancer have developed to the middle and late stage at the time of treatment, and missed the best clinical treatment opportunity. With the continuous development of diagnostic techniques, serum tumor marker detection methods are gradually applied in the detection of gastric cancer patients. At present, the detection samples of gastric cancer tumor markers mainly include tissues, serum, gastric juice, etc., and serum markers such as CA72-4, CA199, CEA, and vascular endothelial growth factor (VEGF) are most widely used in clinical applications (8). There are dozens of valuable tumor markers found in the clinic, but no specific tumor markers have been found

that can achieve specific sensitivity and can meet the early screening of gastric cancer. Therefore, the use of tumor markers for joint detection has important research value on early clinical screening of gastric cancer (9).

DPYSL3, first known as Collapsin response mediator protein 4 (CRMP4), is located on 5q32 and encodes a 62kDa protein, widely expressed in brain tissue and pineal gland. Recent studies have also found it to be moderately expressed in gastric mucosa (10). For patients with malignant tumors, they are expressed in tumor tissues and are considered to be prognostic molecular markers for liver cancer, prostate cancer and gastric cancer. As a specific cell adhesion molecule, most scholars believe that it is one of the important regulators of tumor cell metastasis (11). However, in prostate cancer and pancreatic cancer tissues, there have been conflicting results in the study. In prostate cancer tissues, the concentration of DPYSL3 protein is decreased and correlated with the prognosis of patients, while in pancreatic cancer and gastric cancer, the concentra-

tion of DPYSL3 protein is significantly increased and related to the prognosis of patients. DPYSL3 promotes adhesion and migration of pancreatic cancer cells in vitro as well as metastasis in vivo by activating other cell adhesion genes, thereby facilitating cancer cell invasion and metastasis (12).

In this study, the concentration of DPYSL3 in serum was detected by ELISA. The serum concentration of DPYSL3 in gastric cancer patients was significantly higher than that in healthy controls. The results were consistent with the report (5). It was found by immunohistochemistry that the expression of DPYSL3 protein in cancer tissues was significantly higher than that of normal tissues, and the concentration of DPYSL3 protein mRNA in serum was significantly increased.

This study found that the difference in serum DPYSL3 concentration in patients with different stages and differentiated gastric cancer was significant, that is, the higher the TNM stage, the lower the degree of differentiation, the higher the concentration of DPYSL3 in serum, suggesting that there is a correlation between serum DPYSL3 concentration and malignant degree of gastric cancer. In the progressive gastric cancer and gastric cancer cell lines, especially in gastric cancer cell lines with lymph node metastasis, increased DPYSL3 protein synthesis was observed, which might be associated with increased DPYSL3 protein and increased invasion and metastasis of gastric cancer (13). The precise mechanism still needs further research. This study found that the larger the tumor diameter, the higher the serum DPYSL3 concentration, suggesting that there is a correlation between serum DPYSL3 concentration and tumor burden. The above results suggest that serum DPYSL3 can be used as a marker for early diagnosis of gastric cancer, but the mechanism remains to be further studied.

In this study, the diagnostic value of serum DPYSL3 concentration, CA72-4 and CEA concentration in gastric cancer was obtained by ROC curve analysis. The area under the ROC curve (AUC) of serum DPYSL3 concentration was higher than CA72-4 and CEA. CA72-4 is an early serum marker of gastric cancer discovered in re-

cent years. It is a high molecular glycoprotein antigen mainly found in gastrointestinal adenocarcinoma tissues. It is rarely contained in normal tissues or benign tumors, and its serum level has a certain correlation with the degree of gastric cancer lesions (14). CEA is the most common tumor marker in the clinic. Although it is less specific for the diagnosis of gastric cancer, the analysis shows that it is a serum marker for monitoring postoperative recurrence and prognosis of gastric cancer. This study found that the sensitivity and specificity of CA72-4 concentration as a serodiagnosis index for gastric cancer is higher than that of CEA, suggesting that CA72-4 concentration has more clinical diagnostic value than CEA, which is consistent with previous studies (15), in which CA72-4 was considered more specific in the diagnosis of gastric cancer.

This study showed that the sensitivity and specificity of serum DPYSL3 concentration diagnosis were better than CA72-4 and CEA, suggesting that serum DPYSL3 concentration has a high clinical value for early diagnosis of gastric cancer, and can be used as a new serum-assisted indicator for the diagnosis of gastric cancer.

This study has the following limitations. First, this study is a single-center study with a small number of cases, and a multi-center large sample study is needed to further validate this conclusion. Second, this study did not explore the precise mechanism of increased concentration of serum DPYSL3 in gastric cancer patients, which requires further clinical research.

Conclusion

The serum DPYSL3 concentration in patients with gastric cancer is significantly higher than that in healthy controls. Serum DPYSL3 concentration, CA72-4 and CEA have certain diagnostic value for gastric cancer, and are serum markers of gastric cancer, the mechanism needs further study.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

No funding was received.

Conflict of interest

The authors declare that they have no competing interest.

References

1. Zhang Y, Zheng T, Zhang W (2018). Report of cancer incidence and mortality in China, 2012. *Adv Mod Oncol Res,* 4: 1-7.
2. Khanderia E, Markar SR, Acharya A, et al (2016). The Influence of Gastric Cancer Screening on the Stage at Diagnosis and Survival. *J Clin Gastroenterol,* 50(3): 190-197.
3. Kanda M, Nomoto S, Oya H, et al (2014). Dihydropyrimidinase-like 3 facilitates malignant behavior of gastric cancer. *J Exp Clin Cancer Res,* 33(1): 66.
4. Kanda M, Kodera Y (2015). Recent advances in the molecular diagnostics of gastric cancer. *World J Gastroenterol,* 21(34): 9838-52.
5. Kanda M, Kodera Y (2016). Molecular mechanisms of peritoneal dissemination in gastric cancer. *World J Gastroenterol,* 22(30): 6829.
6. Mocellin S, Verdi D, Pooley KA, Nitti D (2015). Genetic variation and gastric cancer risk: a field synopsis and meta-analysis. *Gut,* 64(8): 1209-1219.
7. Ono H, Yao K, Fujishiro M, et al (2016). Guidelines for endoscopic submucosal dissection and endoscopic mucosal resection for early gastric cancer. *Dig Endosc,* 28(1): 3-15.
8. Shimizu D, Kanda M, Kodera Y (2018). Review of recent molecular landscape knowledge of gastric cancer. *Histol Histopathol,* 33(1): 11-26.
9. Abbas M, Faggian A, Sintali DN, et al (2018). Current and future biomarkers in gastric cancer. *Biomed Pharmacother,* 103: 1688-1700.
10. Matsunuma R, Chan DW, Kim BJ, et al (2018). DPYSL3 modulates mitosis, migration, and epithelial-to-mesenchymal transition in claudin-low breast cancer. *Proc Natl Acad Sci U S A,* 115(51): E11978-1187.
11. Guo H, Xia B (2016). Collapsin response mediator protein 4 isoforms (CRMP4a and CRMP4b) have opposite effects on cell proliferation, migration, and invasion in gastric cancer. *BMC Cancer,* 16: 565.
12. Oya H, Kanda M, Sugimoto H, et al (2015). Dihydropyrimidinase-like 3 is a putative hepatocellular carcinoma tumor suppressor. *J Gastroenterol,* 50(5): 590-600.
13. McFarlane M, Brettschneider J, Gelsthorpe A, et al (2018). An assessment of candidate genes to assist prognosis in gastric cancer. *J Gastrointest Oncol,* 9(2): 303.
14. Liang Y, Wang W, Fang C, et al (2016). Clinical significance and diagnostic value of serum CEA, CA19-9 and CA72-4 in patients with gastric cancer. *Oncotarget,* 7(31): 49565-73.
15. Chen C, Chen Q, Zhao Q, et al (2017). Value of combined detection of serum CEA, CA72-4, CA19-9, CA15-3 and CA12-5 in the diagnosis of gastric cancer. *Ann Clin Lab Sci,* 47(3): 260-263.