Application of serum gastric function markers and digestive tumor indices to the diagnosis of early gastric cancer and precancerous lesions

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ABSTRACT

الأهداف: لدراسة مستويات المستضد السرطاني المضغي (CEA)، ومستضد الكربوهيدرات (CA)، و CA724، CA242، CA199، و البيبسينوجين (PG) الاول والثاني، الجاسترين–17 (G–17)، نسبة PG الاول والثاني (PGR)، في المرضى الذين يعانون من سرطان المعدة المبكر و أورام intraepithelial ولتوفير علامات جديدة لتشخيص سرطان المعدة المبكر والآفات السرطانية.

المنهجية: دراسة بأثر رجعي حيث تم اختبار تركيز مصل الدم لـ CEA و CA199 و CA724 و CA242 و PGI و PGII و G–17 و PGR وأيضًا تم اكتشاف الصيغة البروتينية لـ p27 و Ki67 في أنسجة المرضى عن طريق الكيمياء المناعية في مركز التنظير المعدي المعوي في المستشفى التابع لجامعة Xuzhou الطبية في 20 مارس 2018.

النتائج: اختلفت مستويات مستضد الكربوهيدرات 242 و CA199 في أنسجة الورم بشكل كبير بين المجموعات. انخفضت مستويات البيبسينوجين 1 مع زيادة شدة المرض، وزادت مستويات G–17 مع تفاقم الشدة، وانخفضت الصيغة p27 مع الشدة.

الخلاصة: يمكن أن تكون مجموعة مؤشرات وظائف المعدة في المصل (PGI و G–17) ومؤشرات الورم الهضمي p27 بمثابة علامات لتشخيص سرطان المعدة المبكر وأورام intraepithelial.

Objectives: To study the levels of carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 199, CA724, CA242, pepsinogen (PG) I, PGII, gastrin-17 (G-17), the PGI/PGII ratio (PGR), as well as the expression of p27 and Ki67, in patients suffering from early gastric cancer and intraepithelial neoplasia and to provide new markers for the diagnosis of early gastric cancer and precancerous lesions.

Methods: A retrospective study where the blood serum concentration of CEA, CA199, CA724, CA242, PGI, PGII, G-17 and PGR were tested and also the protein expression of p27 and Ki67 was detected in patients tissues by immunohistochemistry in the Gastrointestinal Endoscopy Center of the Affiliated Hospital of Xuzhou Medical University, Xuzhou, China, from March 2018 to March 2021.

Results: Carbohydrate antigen 242 and CA199 levels in tumor tissue significantly differed among the groups. Pepsinogen I levels decreased with increasing disease severity, G-17 levels increased with the aggravation of severity, and p27 expression decreased with the severity.

Conclusion: The combination of serum gastric function markers (PGI and G-17) and p27 digestive tumor indices can serve as markers for the diagnosis of early gastric cancer and intraepithelial neoplasia.

Keywords: gastric cancer, precancerous lesions, gastro-pannel serum markers

Gastric carcinoma is a major public health issue, as the second cancer cause for death, especially in many Asian countries, such as China, Japan, and Korea. Up to now, as a multi factional disease, containing genetic-infectional, diet-related and environmental factors, the mechanism have not been clearly elucidated.[1,2] Early gastric cancer (EGC) is typically asymptomatic, leading to most diagnosed gastric carcinoma which is advanced gastric cancer (AGC), it has high mortality. Therefore, early diagnosis and treatment is very important to

reduce gastric carcinoma mortality. The detection of gastric neoplasm at early stage is urgent.

Nowadays, these detected bio-markers are mostly suggested for advanced gastric cancer and EGC. On the other hand, precancerous lesions lack relatively specific screening markers. Clinical screening indicators include serum tumor markers and gastro-pannel serum markers. Commonly used tumor markers include carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), CA724, and CA242, which can predict the stage of related tumors.[3]

Pepsinogens (PGs), the precursor of pepsin with no bio-activity, due to different biological properties, are sub-divided into 2 distinct immuno-chemical groups: PGI and PGII. Pepsinogen I is mainly secreted by the chief cells and mucous neck cells from the fundic gland without atrophy. Pepsinogen II are produced by pyloric and Brunner gland cells except chief cell and neck cells.[4] Gastrin-17 (G-17) is exclusively secreted by the G cells from gastric antrum and duodenum. Low concentration of the stimulated G-17 (<3 pmol/L) indicates the presence of antrum atrophic gastritis. Meanwhile, serum gastric function includes serum PGI, PGII, PGI/PGII ratio (PGR), and G-17 combined with the presence of *Helicobacter pylori* (*H. pylori*). Pepsinogen I concentrations below 70 mg/L and PGR less than 3.0 are common cut-offs for identifying atrophic gastritis.[4,5]

The p27 gene, located on chromosome 12p13, is an inhibitor of the CDK2/cyclin E complex, which stalls the cell cycle in G1 phase. Reduced p27 expression is often significantly associated with higher-severity gastric cancer, tumor invasion depth, and lymph node metastasis.[6] The p27 protein as an indicator for negative regulation of cell proliferation, has been less studied in EGC and precancerous lesions. The Ki-67 antigen locating in the nucleus is expressed in the S, G1, G2, and M phases of the cell cycle, which is a nuclear division and proliferation related protein and is often used as a reliable marker of tumor cell proliferation activity. However, other studies have not shown the expression of p27 and Ki67 in EGC and pre-carcinous condition. The requirement for a non-invasive approach to detect EGC is urgent, now researchers have obtained more attention on serum PGs evaluation and these carcinomous bio-markers.

In our study, we analyzed the concentrations of CA724, CEA, CA242, and CA199. We also detected PGI, PGII, and G-17 levels, PGR, and *H. pylori* infection in patients with precancerous lesions and EGC to identify diagnostic indices for early gastric mucosal lesions.

Methods. This is a retrospective study. All enrolled 131 patients had undergone endoscopic submucosal dissection (ESD) and been pathologically confirmed as having gastric intraepithelial neoplasia (IN) or differentiated EGC in the Gastrointestinal Endoscopy Center of the Affiliated Hospital of Xuzhou Medical University, Xuzhou, China, from March 2018 to March 2021. Among them, 63 patients had EGC, 25 patients had high-grade IN (HGIN), and 43 patients had low-grade IN (LGIN). The positive control group consisted of 40 patients who underwent surgery for AGC. For the negative control, 40 healthy subjects selected underwent endoscopy without abnormalities within the same time period. The inclusion criteria were patients aged 18-80, with differentiated EGC and precancerous lesions. While the exclusion criteria were i) patient unable to cooperate or tolerate gastroscopy, ESD surgery, or refuses to sign consent form; ii) patients with undifferentiated or mixed type gastric cancer, or residual gastric cancer.

The concentrations of CEA, CA242, CA199, CA724, PGI, PGII, and G-17 were measured in each participant's serum. The Ki67 and p27 were detected by immunohistochemistry. In this analysis, 20 patients with AGC tissue were selected as the positive control group. According to principles of Helsinki Declaration, the subjects underwent digestive tract tumor marker and serum gastric function examinations upon admission. The study was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University, Xuzhou, China (no.: XYFY2020-KL045-01).

The levels of G-17, PGI, and PGII were determined by enzyme-linked immunosorbent assay. Carcinoembryonic antigen 242, CA724, and CA199 levels were detected by chemiluminescence immunoassay. We carried out the experimental procedures strictly based on the instructions of the kits. The results were recorded in the experimental record book.

For p27 and Ki67, the results were separately read by 2 senior pathologists. The p27 and Ki67 were stained in the nucleus, which was brown and granular. Then, 5-10 high-fold visual fields were examined, and the quantity of positive cells among 100 tumor cells was counted and expressed as percentage. The Ki67 staining

was scored as follows: <10% (negative); 10-20% (+); 20-50% (++); and >50% (+++). Meanwhile, p27 expression was graded as follows: <10%; 10-20%; 20-30%; and >30%. Abnormal p27 expression was indicated by a positivity rate of less than 20%, and the abnormal expression rate was scored in each group.

The CEA, CA242, CA724, and CA199 kits were obtained from Roche (Switzerland). The PGI, PGII, and G-17 kits were obtained from Enzyme Union Biotech Co. (Shanghai, China). Rabbit anti-human Ki67 and rat anti-human p27 antibodies were purchased from Zhongshan Jinqiao Biotechnology Co. Ltd. (Beijing, China).

Statistical analysis. The Statistical Package for the Social Sciences, version 19.0 was used. Measurement data were expressed as the mean ± standard deviation (SD), and comparisons between groups were carried out using ANOVA. Countable data are presented as numbers and percentages. The Chi-squared test was used for comparisons. A *p*-value of <0.05 was considered significant.

Results. As shown in Table 1, CEA and CA724 levels did not differ among the 5 groups . However, CA242 and CA199 levels significantly differed among the 5 groups (*p*<0.05). Compared with the normal control group and LGIN group, CA242 and CA199 in AGC group was higher than the 2 ones, CA242 and CA199 levels did not differ among the LGIN, HGIN, and EGC groups, implying that these 2 indicators have limited value for differentiating LGIN, HGIN, and EGC, but they have predictive significance for AGC.

Pepsinogen I and G-17 significantly differed among the groups (*p*<0.05), and PGI levels were additionally different among the LGIN, HGIN, and EGC groups (*p*<0.05). In general, PGI tended to decrease with

increasing lesion severity. Meanwhile, G-17 levels differed among the LGIN, HGIN, and EGC groups (*p*<0.05). Specifically, G-17 levels increased with lesion severity. Pepsinogen II levels and PGR did not differ among the groups (Table 2).

In the LGIN, HGIN, and EGC groups, PGI and PGII levels were higher in *H. pylori*-positive patients than in *H. pylori*-negative patients (*p*<0.05). Meanwhile, G-17 levels did not differ between *H. pylori*-negative and *H. pylori*-positive patients (*p*>0.05; Table 3)

As presented in Table 4, the Ki67 positivity rate gradually increased with disease severity (*p*<0.05). Conversely, the positive rate did not differ among LGIN, HGIN, and EGC groups (Table4).

As presented in Table 5, in the negative control group, the proportion of patients with abnormal expression of p27 was only15%, in LGIN the proption was 13.9% and in HGIN the proption was 32%. Whereas in the EGC and AGC groups, the proportion increased to 69.8-75%. In the comparison of the abnormal expression rate among LGIN, HGIN, and EGC, the difference was significant among the groups (*p*<0.05), and the rate increased with the aggravation of severity (Table 5).

Discussion. With the the development of Chinese society and economy, the incidence of gastric cancer is tending to increase in younger patients.[7] Because of the poor long-term prognosis and survival rate of AGC, early diagnosis and treatment for gastric cancer are urgently required. Pepsinogen I is produced by chief cells. In addition to these cells, PGII is also produced by pylorus and Brenner gland cells. These zymogens are mainly excreted into the stomach cavity, and the small proportion (approximately 1%) that returns to blood can be measured.[8] Some Western countries and Asian

Table 1 - Expression of tumor indicators in different lesions.

Groups	Number of cases	Tumor indicators			
		CEA (ng/ml)	CA242 (u/ml)	CA724 (u/ml)	CA199 (u/ml)
Normal	40	2.1±0.79	4.52±1.34	2.14±0.42	11.01±2.57
LGIN†	43	2.33±0.93	4.24±1.13	2.30±0.67	10.24±1.96
HGIN†	25	2.41±0.67	7.37±2.01	2.92±0.89	11.38±2.39
EGC†	63	2.86±1.09	6.97±2.31	3.61±1.03	17.25±4.17
AGC†	40	3.4±0.85	17.36±3.97	3.17±1.04	34.19±5.74
P-values		*p*>0.05*	*p*<0.05*	*p*>0.05*	*p*<0.05*
		p>0.05†	*p*>0.05†	*p*>0.05†	*p*>0.05†

Values are presented as mean ± standard deviation (SD). *comparison among all groups, †comparison among the LGIN, HGIN, and EGC groups. CEA: carcinoembryonic antigen, CA: carbohydrate antigen, LGIN: low-grade intraepithelial neoplasia, HGIN: high-grade intraepithelial neoplasia, EGC: early gastric cancer, AGC: advanced gastric cancer

Table 2 - Pepsinogen I, pepsinogen II, and gastrin-17 levels in early gastric cancer and intraepithelial neoplasia.

Groups	PGI	PGII	PGR	G-17
Control	143.15±21.26	16.87±3.61	10.89±1.49	10.05±2.59
LGIN[†]	187.21±25.73	18.95±3.96	12.33±2.82	14.26±3.47
HGIN[†]	146.16±17.48	17.76±4.71	13.19±3.30	18.66±4.82
EGC[†]	119.35±25.52	20.55±4.57	11.24±2.75	21.72±5.58
Advanced cancer	94.85±28.72	17.3± 5.23	9.83±2.67	22.38±5.35
P-values	$p<0.05^*$	$p>0.05^*$	$p>0.05^*$	$p<0.05^*$
	$p<0.05^†$	$p>0.05^†$	$p>0.05^†$	$p<0.05^†$

Values are presented as mean ± standard deviation (SD). *comparison among all groups, †comparison among the LGIN, HGIN, and EGC groups. PG: pepsinogen, PGR: PGI/PGII ratio, G-17: gastrin-17, LGIN: low-grade intraepithelial neoplasia, HGIN: high-grade intraepithelial neoplasia, EGC: early gastric cancer

Table 3 - Indices of serum gastric function in *Helicobacter pylori*-infect`ed and uninfected patients.

Groups	PGI				PGII				G-17			
	H. pylori (+)	P-value	*H. pylori* (-)	P-value	*H. pylori* (+)	P-value	*H. pylori* (-)	P-value	*H. pylori* (+)	P-value	*H. pylori* (-)	P-value
LGIN	238.51±25.48	<0.05	157.82±14.78	<0.05	27.6±3.57	<0.05	14.05±2.01	<0.05	10.98±1.74	>0.05	13.83±2.35	>0.05
HGIN	171.13±21.46		109.00±11.25		22.70±4.82		16.97±3,14		13.49±2.85		16.13±3.48	
EGC	130.82±18.37		103.47±9.87		13.69±2.18		9.83±1.78		22.51±3.94		20.41±4.06	

Values are presented as mean ± standard deviation (SD). PG: pepsinogen, G-17: gastrin-17, *H. pylori: Helicobacter pylori*, LGIN: low-grade intraepithelial neoplasia, HGIN: high-grade intraepithelial neoplasia, EGC: early gastric cancer

Table 4 - Expression of Ki67 among the groups.

Groups	Cases	Ki67						P-values	P-values*
		Negative	+	++	+++	Positive	Positive rate (%)		
Negative control	20	14	4	2	0	6	30.0		
LGIN	43	23	16	4	0	20	46.5		
HGIN	25	11	11	3	0	14	56.0		
EGC	63	17	13	25	8	46	73.0		>0.05
AGC	20	3	2	8	7	17	85.0	<0.05	

LGIN: low-grade intraepithelial neoplasia, HGIN: high-grade intraepithelial neoplasia, EGC: early gastric cancer, AGC: advanced gastric cancer, P: comparison among these groups. P*: comparison among LGIN, HGIN, early gastric cancer

countries such as Japan and South Korea use serological indicators such as PG, PGR, and G-17 to screen for EGC. In this strategy, patients who are negative for these indicators are exempted from gastrointestinal endoscopy, which has negative predictive significance but a certain rate of missed diagnosis.[9,10]

The detection of *H. pylori* infection combined with PGI, PGII, PGR, and G-17 can assess the condition of gastric mucosal atrophy and predict the risk of EGC. Studies by Miki[11,12] and other researchers found that when PGI is ≤70 ng/mL and PGR is ≤3, they were selected as the cut-offs, sensitivity and specificity of atrophic diagnosis are high, and atrophic gastritis, intestinal metaplasia, atypical hyperplasia, and other high-risk states can be diagnosed.

As suggested in this study, PGI levels decreased with disease severity (LGIN, HGIN, EGC, and AGC). Gastrin-17 levels increased with increasing disease severity. Pepsinogen II levels and PGR did not differ among the groups. The reason for these findings might be that with the aggravation of dysplasia, mucosal destruction, and atrophy of gastric body inflammation, the destruction and atrophy of chief cells increase, resulting in decreased PGI secretion, whereas PGII can be produced in other glands with little change. Within the same group, the levels of PGI and PGII were higher in *H. pylori*-positive patients than in *H. pylori*-negative patients, indicating that *H. pylori* infection might stimulate glands to secrete PGI and PGII. Research has found that PGII has a strict correlation with chronic

Table 5 - Abnormal p27 expression rate in each group.

Groups	Cases	p27				Abnormal expression rate (%)	*P*-values	*P*-values*
		<10%	10-20%	21-30%	30% above			
Negative control	20	1	2	0	17	15.0		
LGIN	43	3	3	0	37	13.9		
HGIN	25	4	4	17	0	32.0		
EGC	63	34	10	5	14	69.8		<0.05
AGC	20	11	4	4	1	75.0	<0.05	

LGIN: low-grade intraepithelial neoplasia, HGIN: high-grade intraepithelial neoplasia, EGC: early gastric cancer, AGC: advanced gastric cancer, P: comparison among these group, P*: comparison among LGIN, GHIN, ECG

gastritis caused by *H. pylori* infection.[13] The maximum acid output is positively correlated with the PGI level and negatively correlated with gastric atrophy and inflammation. In the same group, there was no significant difference in serum G-17 levels between *H. pylori*-positive and *H. pylori*-negative patients. Considering that the main influencing factor of G-17 secretion is gastric acid, its secretion can be stimulated only when the concentration of gastric acid is significantly reduced, whereas the main pathological process caused by *H. pylori* infection is acute or chronic inflammation of the mucosal epithelium. Inflammation must occur in a sequential order to cause parietal cells to secrete gastric acid, and the stimulation of gastric acid secretion requires multiple mechanisms.[14,15] In a recent study, researchers have shown *H. pylori* infection contributed to NAT10 induction, which can promoted cellular G2/M phase progression, proliferation, and tumorigenicity of GC.[16] Another study revealed the advances of tRFs in GC and their functions in gene expression regulation and the related signal transduction pathways associated with them.[17] On the basis of the previous and present study, we believe that PGI and G-17 are valuable for differentiating EGC and gastric IN. However, the small number of samples and operator error might have influence on the accuracy of the result. Therefore, the research sample must be increased in further research.

Tumor markers are metabolic substances such as antigens and enzymes produced by tumor cells in the procession of proliferation. Clinically, tumors are identified and diagnosed according to biochemical and immune tumor indicators in different tissues, and these indicators reflect the activity of tumor metabolism.[18]

In this study, CEA, CA242, CA724, and CA199 levels in IN, EGC, and AGC were jointly detected. The results revealed no significant difference in CEA and CA724 levels among these groups, whereas CA242 and CA199 levels significantly differed among these groups. Carbohydrate antigen 242 and CA199 levels did not differ among the LGIN, HGIN, and EGC groups, but their levels increased with disease severity. Previous research revealed that CEA is useful for the follow-up of patients with EGC.[19,20] Carbohydrate antigen 724 have low sensitivity and poor diagnostic utility in EGC, which is basically consistent with our findings. In our study, CEA and CA724 levels did not differ among the groups. It is believed that these 2 indicators have limited diagnostic value in EGC screening. According to prior research in other countries, the CEA positivity rate in EGC is less than 30%, whereas the reported rate in China is 37.7%.[21,22] We believe that the combination of CA242, CA199, PGI, and G-17 might improve the diagnostic accuracy of EGC screening.

Kim et al[23] found that low p27 expression is relevant to tumor prognosis, lymph node metastasis, and cell proliferation. The current study suggested that the p27 positivity rate was lower in the advanced cancer group than in the EGC group, and p27 expression decreased with disease aggravation. In addition, the p27 positivity rate significantly differed among the LGIN, HGIN, and EGC groups. Some studies reported that the p27 positivity rate was significantly higher in precancerous lesions than in EGC and AGC.[24] Our study only explored the relative expression of proteins in LGIN, HGIN, and EGC. Additional research is needed to detect the expression of the p27 gene and clarify its expression at the cellular and molecular levels.

The occurrence of gastric cancer is a multifactorial and multistep process. Under the stimulation of various factors, the gastric mucosal epithelium undergoes inflammatory reaction, which leads to atrophy, intestinal metaplasia, and repeated hyperplasia and dysplasia, eventually transforming this tissue into neoplastic epithelium.[25] In the process of transformation into neoplastic epithelium, early identification of IN, especially HGIN and EGC, can contribute to achieve early diagnosis, and early treatment. Timely intervention is needed for HGIN and EGC because these 2 types of lesions are at risk of distant metastasis and invasive

growth, Which is associated with tumor growth patterns and biological characteristics such as p53 and tumor angiogenesis according to some references.[26,27] In the diagnosis of EGC, tumor indicators with high sensitivity and specificity should be selected and combined with PGI and G-17 to improve the diagnostic accuracy.

Study limitations. Because of the limitations of small sample size in our study, a prospective large sample study is needed for validation. Our findings may offer researchers new ideas for cancer treatment as well as potential biomarkers for further research in GC.

In conclusion, this study found CA242, CA199, PGI, and G-17 might improve the diagnostic accuracy in EGC screening. Additionally, p27 and Ki67 are useful in the diagnosis of EGC and precancerous lesion.

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