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ORIGINAL ARTICLE

Investigation on correlations of serum IL-26 with diagnosis and staging of gastric cancer

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Summary

Purpose: *To investigate the correlations of interleukin-26 (IL-26) with the diagnosis and pathological stage of gastric cancer by comparing the serum IL-26 levels in patients with different pathological stages of gastric cancer.*

Methods: *A total of 302 patients with gastric cancer hospitalized in Chinese PLA General Hospital from April 2015 to October 2017 were selected and divided into 4 groups according to the seventh edition of the staging guidance for gastric cancer of American Joint Committee on Cancer (AJCC), including stage I (n=75), stage II (n=73), stage III (n=125) and stage IV (n=29). Meanwhile, patients with benign stomach diseases admitted to the hospital during the same period were enrolled as controls (n=100). The differences in the levels of IL-26 and tumor markers between benign stomach diseases and gastric cancer and among stages were compared, and the correlations of IL-26 with the occurrence and different pathological stages of gastric cancer were analyzed.*

Results: *The serum IL-26 level in the patients with gastric cancer was remarkably higher than that in benign stomach disease group [(267.14±20.39) vs. (172.12±13.38) pg/mL, p<0.05], which was similar to the change trends of tumor markers such as carbohydrate antigen (CA) 724. The serum IL-26 level in the gastric cancer tissues was gradually elevated with the increase in clinical stage of gastric cancer [stage I: (213.05±19.05) pg/mL, stage II: (244.98±24.14) pg/mL, stage III: (278.45±20.68) pg/mL and stage IV: (291.73±15.62) pg/mL; p<0.05]. The serum IL-26 level had positive correlations with gastric cancer (r=0.528, p<0.001). Logistic regression analysis indicated that serum IL-26 was still an independent risk factor for the occurrence of gastric cancer after age, gender, carcinoembryonic antigen (CEA), CA125 and other risk factors were adjusted [odds ratio (OR)=1.216, P=0.002].*

Conclusions: *The serum IL-26 level is closely correlated with gastric cancer and its clinicopathological stages, which is of important value for determination of the occurrence and development of the disease.*

Key words: *IL-26, gastric cancer, diagnostic value, pathological stage*

Introduction

Gastric cancer is one of the common malignant tumors in the digestive tract, and most patients have been in the advanced stage when diagnosed according to statistical research in China [1,2]. Therefore, it is necessary to apply sensitive detection indicators for early diagnosis and monitoring of the disease. There are plenty of mechanisms for the occurrence and development of gastric cancer. It has been proven that the chronic inflammations induced by immune responses which are centered on virulence cytokines produced by Helicobacter pylori and helper T-cell 17 (Th17) play key roles in the mechanisms, including retinoid-related orphan receptor C (RORC), interleukin-1 (IL-1), IL-17 and IL-23 [3-5]. Among them, the lowly expressed RORC can promote the occurrence, development and differentiation of gastric cancer. The highly expressed IL-17 can accelerate the formation of new

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blood vessels in tumors, thus affecting the tumor growth [4]. The highly expressed IL-1 and IL-23 can promote tumor metastasis [5]. IL-26 exerts regulating effects on multiple chronic inflammations and autoimmune diseases, which is closely related to tumor proliferation, invasion, migration and immune escape [6-7]. Currently, there are few reports on IL-26 generated by Th17 cells and gastric cancer, so this research aims to investigate the value of IL-26 in the diagnosis of gastric cancer and the correlation of IL-26 with different stages of gastric cancer.

Methods

Subjects

A total of 100 patients with benign gastric diseases and 302 patients with gastric cancer hospitalized in Chinese PLA General Hospital from April 2015 to October 2017 were selected as research objects. All enrolled patients were definitely diagnosed via pathological examinations. Inclusion criteria: patients had no contraindications for radiotherapy, severe cardiac insufficiency and abnormal liver and kidney function. Patients who were unconscious or had mental disorders, hematological disorders, systemic infectious diseases, communicable diseases, autoimmune diseases or other malignant tumors were excluded.

In the benign gastric disease group there were 39 males and 61 females aged 40-65 years, (mean 52.84±7.79), including 27 cases of gastritis, 58 cases of gastric polyp and 15 cases of gastric ulcer. In the gastric cancer group, there were 234 males and 68 females aged 39-64 years (mean 50.36±7.25). Based on the postoperative pathological findings, the patients with gastric cancer were divided into 4 groups according to the seventh edition of the staging criteria for gastric cancer of American Joint Committee on Cancer (AJCC) [8], including stage I (n=75), stage II (n=73), stage III (n=125) and stage IV (n=29). The above diagnoses were histopathologically confirmed, and none of the patients with gastric cancer received chemoradiotherapy. Signed written informed consent was obtained from all participants before this research. This study was approved by the ethics committee of Chinese PLA General Hospital.

Methods

Specimen collection: All the participants underwent routinely fasting for 12 hrs, so as to reduce and eliminate the stimulation on the vagus nerve or gastric antrum as much as possible. About 5 mL fasting antecubital venous blood was collected from the patients with gastric cancer before operation into an anticoagulant tube, which was mixed immediately, followed by standing at room temperature for 2 hrs, centrifugation at 3000 rpm and normal temperature for 15 min. Then the supernatant was taken and stored at -80°C for subsequent experiments. The serum of the patients with benign stomach diseases was collected using the same method.

Specimen detection: Enzyme-linked immunosorbent assay (ELISA) was performed to measure the concentrations of serum IL-26 and tumor markers such as carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 199, CA125, CA724 and alpha fetoprotein (AFP). IL-26 kit was purchased from Cloud-Clone Corp., USA, and C8000 biochemical analyzer (Roche, Germany) was used as the detection instrument. The specific detection steps were in line with the instructions of the instrument and reagent.

Statistics

All statistics were performed using SPSS 20.0 software (IBM, Armonk, NY, USA). Quantitative data in normal distribution were presented as mean ± standard deviation (x±s), and classification data were expressed as rate or frequency. Inter-group rate or composition ratio was compared with chi-square test, *t*-test was conducted to compare differences between the benign stomach disease group and the gastric cancer group, and multi-group data were compared using analysis of variance. The correlation of various factors with the stage of gastric cancer was analyzed by Spearman correlation analysis. Binary logistic regression analysis was applied to evaluate the risk factors of gastric cancer. $P<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of general characteristics and laboratory indexes

There were no statistically significant differences in age, biochemical indexes [aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and free fatty acid (FFA)] and related indexes to renal function [blood urea nitrogen (BUN) and creatine (Cr)] among the 5 groups of patients ($p>0.05$, Table 1). The difference in sex constituent ratio was statistically significant among the groups ($p<0.001$), but it was not significant between benign stomach disease group and gastric cancer group and among the 4 gastric cancer groups ($P=0.517$). The differences in biochemical indexes [alanine aminotransferase (ALT), alkaline phosphatase (AKP), total cholesterol (TC) and glucose (GLU)] were statistically significant among the 5 groups ($p<0.05$, Table 1).

Comparisons of levels of serum IL-26 and tumor markers

There was no statistically significant difference in the serum AFP level among the 5 groups ($p=0.346$, Table 2). However, the differences in serum IL-26, CEA, CA199, CA125, CA724 and ferritin levels were statistically significant among the 5 groups ($p<0.05$, Table 2). The serum IL-26 level

was gradually elevated with the increase in severity of stomach diseases (p<0.001, Table 2), which was the lowest in benign stomach disease group [(172.12±13.38) pg/mL] and the highest in stage IV gastric cancer group [(291.73±15.62) pg/mL].

Analysis on correlations of IL-26 and tumor marker levels with gastric cancer stages

IL-26, CA199, CA125, CA724 and CEA were positively correlated with the severity of stomach diseases (r=0.528, 0.236, 0.197, 0.285, 0.314, p<0.05), ferritin was negatively correlated with the severity of stomach diseases (r=-0.329, p=0.015), and AFP had no significant correlation with the stage of gastric cancer (p>0.05, Table 3).

Logistic regression analysis results

With the occurrence of gastric cancer as the dependent variable, binary Logistic regression analysis was conducted, and the age, gender and tumor markers were adjusted at the same time. The results manifested that the increased IL-26 level was an independent risk factor for the occurrence of gastric cancer [odds ratio (OR) =1.216 (1.044-1.379), p=0.002]. The occurrence of gastric cancer was related to age [OR=0.935 (0.878-0.992), p=0.025], gender [OR=0.168 (0.062-0.357), p<0.001] and ferritin [OR=0.994 (0.979-0.998), p=0.002], while other tumor markers (CA199, CA724, CA125, CEA and AFP) had no statistical correlations with the occurrence of gastric cancer (p>0.05, Table 4).

Table 1. Baseline characteristics of enrolled subjects

	Benign stomach disease group (n=100)	*Stage I gastric cancer (n=75)*	*Stage II gastric cancer (n=73)*	*Stage III gastric cancer (n=125)*	*Stage IV gastric cancer (n=29)*	*p*
Male, n(%)	39 (39)	53 (70.67)^{aa}	60 (82.19)^{aa}	99 (79.20)^{aa}	22 (75.86)^{aa}	<0.01
Female, n(%)	61 (61)	22 (29.33)^{aa}	13 (17.81)^{aa}	26 (20.80)^{aa}	7 (24.14)^{aa}	<0.01
Age (y)	52.84±7.79	50.36±7.25	52.45±6.87	51.37±7.01	49.58±7.92	0.225
ALT (U/L)	14.17±0.72	17.25±1.33^{a}	16.26±1.24	13.94±0.81^{b}	13.64±1.63^{b}	0.013
AST (U/L)	18.53±5.17	19.24±4.39	18.65±4.47	17.62±5.08	19.29±3.64	0.315
GGT (U/L)	20.43±2.26	21.72±3.33	19.37±1.35	18.92±1.38	19.45±3.39	0.417
AKP (U/L)	71.22±20.43	70.41±21.23	75.95±18.72	72.84±17.26	40.74±11.28^{abcd}	0.024
TC (mmol/L)	4.51±0.81	4.56±0.72	4.52±0.69	4.33±0.78^{a}	3.96±0.80^{abc}	0.016
TG (mmol/L)	1.11±0.09	1.19±0.21	1.45±0.35	1.09±0.07	0.96±0.08	0.508
HDL (mmol/L)	2.18±1.55	1.34±0.27	1.26±0.29	1.21±0.24	1.12±0.20	0.657
LDL (mmol/L)	2.61±0.71	2.68±0.62	2.72±0.66	2.65±0.63	2.57±0.74	0.786
FFA (umol/L)	40.26±1.98	40.05±2.94	41.69±3.13	42.92±2.38	43.04±4.36	0.839
Glu (mmol/L)	4.70±0.62	4.77±0.45	5.01±0.59^{a}	4.96±0.47^{a}	4.68±0.53^{c}	0.042
BUN (mmol/L)	4.56±1.24	4.89±1.32	5.86±2.15	6.02±2.07	4.63±1.45	0.252
Cr (umol/L)	58.56±10.18	62.57±11.06	66.82±10.07	65.41±11.20	63.49±12.95	0.059

In comparison with benign stomach disease group, ^{a}p<0.05, ^{aa}p<0.01; In comparison with stage I gastric cancer, ^{b}p<0.05; In comparison with stage II gastric cancer, ^{c}p<0.05; In comparison with stage III gastric cancer, ^{d}p<0.05. P<0.05 suggested statistical significance.

Table 2. Serum IL-26 and tumor markers in enrolled patients

	Benign stomach disease group (n=100)	*Stage I gastric cancer (n=75)*	*Stage II gastric cancer (n=73)*	*Stage III gastric cancer (n=125)*	*Stage IV gastric cancer (n=29)*	*p*
AFP (ng/mL)	3.26±0.18	3.68±0.32	4.54±0.53	5.02±1.45	4.78±1.39	0.346
CEA (ng/mL)	1.87±0.24	2.19±0.32	4.93±2.43	9.52±3.51^{a}	30.26±14.78^{aabcd}	<0.001
Ferritin (ng/mL)	140.89±20.15	126.29±17.93	108.84±19.62	75.56±12.59^{ab}	92.64±34.47	0.015
CA199 (U/mL)	10.29±0.97	10.52±1.24	40.36±25.45	89.76±29.37^{ab}	125.79±58.46^{aabbccd}	<0.001
CA125 (U/mL)	8.58±0.85	9.04±0.92	8.97±0.84	11.43±1.26	49.59±18.73^{aabbccdd}	<0.001
CA724 (U/mL)	2.96±0.42	4.78±2.14	3.63±0.53	13.05±5.39^{a}	46.78±23.35^{aabbccdd}	<0.001
IL-26 (ng/mL)	172.12±13.38	213.05±19.05^{aa}	244.98±24.14^{aa}	278.45±20.68^{aabb}	291.73±15.62^{aabbcd}	<0.001

In comparison with benign stomach disease group, ^{a}p<0.05, ^{aa}p<0.01; In comparison with stage I gastric cancer, ^{b}p<0.05, ^{bb}p<0.01; In comparison with stage II gastric cance, ^{c}p<0.05, ^{cc}p<0.01; In comparison with stage III gastric cance, ^{d}p<0.05, ^{dd}p<0.01. P<0.05 suggested statistical significance.

Discussion

IL-26, belonging to type II or IL-10 cytokine family, is mainly produced by Th17 cells, which has a close association with tumors [6,7]. Some authors have pointed out that IL-26 can activate signal transducer and activator of transcription (STAT) protein by activating phosphorylation of Janus tyrosine kinase after the conformation of receptor complex was altered [9]. Moreover, the ability of STAT3 to shift to the nucleus due to constant phosphorylation can promote the occurrence of gastric cancer. The study of Yamada et al. indicated that Th17 cells in the tumor tissues of the patients with gastric cancer are increased obviously compared with those in healthy controls, but Th17 cells in the peripheral blood are not significantly different from those in healthy controls [3]. In addition, they are not related to clinical stage, lymph node metastasis and operation. The IL-17 level in the peripheral blood is elevated markedly compared with that in healthy controls, and it is an independent predictor of the patient's 5-year survival time. These may be the results that cluster of differentiation 4 $(CD4)^+$ T cells in the tumor tissues differentiate into Th17 cells or Th17 cells in the peripheral blood migrate to the tumor tissues under the induction of tumor micro-environment [10,11]. It was confirmed in this research that IL-26 expression in the peripheral blood of patients with gastric cancer was higher than that in benign stomach disease group, which is consistent with the findings of previous studies. The possible reason is that IL-26 expression in the peripheral blood is raised due to the increase in Th17 cells in the patients with gastric cancer. Studies have demonstrated that Th17 cells in the peripheral blood and tumor-infiltrating lymph node of the patients with gastric cancer are increased notably, and the increase is associated with the clinical stage and prognosis. Th17-related cytokines such as IL-17, IL23p19 and RORC are also increased obviously, increased number of Th17 cells in tumor is positively correlated with microvascular density, and IL-17 secreted by Th17 cells can accelerate tumor neovascularization, thus promoting tumor growth [4,5,10,11]. Furthermore, the higher the microvascular density is, the poorer the prognosis will be. Some studies also suggest that the serum IL-26 expression in the patients with gastric cancer is associated with tumor size, but not significantly correlated with age, gender, tumor location, depth of invasion, Lauren's classification, lymph node metastasis and clinical stage [9,12]. Currently, the specific mechanism of IL-26 and gastric cancer is still not very clear, which needs to be further explored.

The pathological stage of gastric cancer plays an important role in the clinical treatment of the disease, which is related to disease assessment and treatment protocol selection, and is closely associated with the patient's prognosis. CA724 has relatively high specificity in diagnosing gastric cancer [13]. In this research, CA724 level in gastric cancer

Table 3. Spearman's analysis between IL-26 and tumor marker levels with gastric cancer stages

	r	*p*
IL-26	0.528	<0.001
CEA	0.314	<0.001
CA199	0.236	0.017
CA125	0.197	0.032
CA724	0.285	<0.001
AFP	0.063	0.414
Ferritin	-0.329	0.015

Table 4. Logistic regression analysis

	Model 1		*Model 2*		*Model 3*	
	OR (95%CI)	*p*	*OR (95%CI)*	*p*	*OR (95%CI)*	*p*
IL-26	1.322 (1.125-1.497)	<0.001	1.268 (1.109-1.436)	<0.001	1.216 (1.044-1.379)	0.002
Age			0.967 (0.902-0.994)	0.018	0.935 (0.878-0.992)	0.025
Gender			0.273 (0.126-0.528)	<0.001	0.168 (0.062-0.357)	<0.001
AFP					1.223 (0.962-1.501)	0.073
CEA					1.178 (0.985-1.496)	0.084
Ferritin					0.994 (0.979-0.998)	0.002
CA199					1.042 (0.993-1.076)	0.216
CA125					0.985 (0.917-1.052)	0.459
CA724					1.064 (0.966-1.147)	0.348

Model 1, with no correction; Model 2, after correction of age and gender; Model 3, after correction of age, gender, AFP, CEA, ferritin, CA199, CA125 and CA724. P<0.05 suggested statistical significance.

group was higher than that in benign stomach disease group. Particularly, CA724 level in stage IV gastric cancer group was remarkably higher than that in other groups, and the increase was more apparent in later pathological stages. The change trends of other tumor markers (CA199, CEA and CA125) were similar to that of CA724, which can reflect the severity of stomach disease in some degree. The comparison of IL-26 level among groups revealed that the change trend of IL-26 concentration among the 4 groups was similar to those of tumor markers, and IL-26 concentration was increased with the advancement of pathological stage, which manifested differences in the early stage of gastric cancer compared with that in benign gastric disease (there was a statistically significant difference in IL-26 level between stage I gastric cancer group and benign gastric disease group). Meanwhile, correlation analysis implied that serum IL-26 is significantly related to gastric cancer stage. Therefore, the application of IL-26 combined with other tumor markers for screening and prognosis assessment of gastric cancer can be considered to guide clinical work. The serum IL-26 concentration in gastric cancer group was included into the binary logistic regression equation for analysis, with that in patients in the benign gastric disease group as the reference standard. At the same time, such factors as age, gender and tumor markers were adjusted. The results showed that the increased serum IL-26 concentration was an independent risk factor for gastric cancer.

In conclusion, the serum IL-26 has similar characteristics to tumor markers in the detection of gastric cancer, which is capable of reflecting the risk of stomach diseases, associated with the pathological stage of gastric cancer and able to serve as a marker of the disease. Meanwhile, the serum IL-26 is also a risk factor for gastric cancer, whose level, however, is influenced by multiple factors and specificity is relatively low, so it is difficult to be used in the detection of gastric cancer alone. It may be combined with other tumor markers for the early diagnosis and condition assessment of gastric cancer, thereby increasing the diagnostic sensitivity. At the same time, considering that the high IL-26 level is a risk factor for gastric cancer, the control and adjustment of IL-26 level can provide new directions for the treatment and prevention of the disease.

There are still some deficiencies in this research that need to be improved in future studies. Firstly, the sample size for research was relatively insufficient, and it was smaller in some subgroups, which could be resolved by enlarging the sample size. Secondly, this research was a cross-sectional study, which could only analyze the statistical correlations among the factors, but could not clarify their causality. Finally, normal controls were not set up in this research due to the sources of research objects, which could be established in subsequent studies.

Conclusions

The serum IL-26 is a risk factor for gastric cancer, which can be considered as a tumor marker for the detection of the disease. It is able to reflect the progression and severity of gastric cancer, thus having certain clinical value for the diagnosis, condition assessment and prognosis judgment of patients with gastric cancer.

Conflict of interests

The authors declare no conflict of interests.

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