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# Clinical Significance of Serum Chemokines in Esophageal Cancer

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**Background:** The aim of this study was to detect the expression levels of chemokines (CX3CL1, CXCL-11, CXCL-12, CCL3, CCL4, and CCL20) in the serum of esophageal cancer patients and a normal control group, and to explore the correlations of those expression levels with the pathological type, progression, and metastasis of esophageal cancer.





**Material/Methods:** A total of 50 normal people and 50 untreated patients initially diagnosed with esophageal cancer (including 17 cases of non-metastatic esophageal cancer, 33 cases of metastatic esophageal cancer, 36 cases of esophageal squamous cell carcinoma and 14 cases of esophageal adenocarcinoma) were collected. The liquid chip (Luminex) technology was applied to detect the expression levels of the above-mentioned serum chemokines in the two groups. The results were analyzed using Statistical Product and Service Solution 20.0 software.

**Results:** The expression levels of CX3CL1, CXCL-12, and CCL20 in esophageal cancer group were evidently higher than those in normal control group ( $P < 0.001$ ,  $P < 0.001$  and  $P = 0.003$ , respectively). There were no statistically significant differences in chemokine expressions between metastatic esophageal cancer group and non-metastatic esophageal cancer group ( $P > 0.05$ ). The expression level of serum CCL4 in esophageal adenocarcinoma group was remarkably higher than that in esophageal squamous cell carcinoma group [18.45 (11.94) versus 13.37 (9.29),  $Z = -2.039$ ,  $P = 0.031$ ]. In esophageal cancer group and normal control group, the serum CX3CL1 was positively correlated with CCL20 ( $r = 0.649$ ,  $P < 0.001$ ,  $r = 0.758$ ,  $P < 0.001$ ).

**Conclusions:** The expressions of serum CX3CL1, CXCL-12, and CCL20 are increased markedly in the patients, which may promote the occurrence, development and metastasis of esophageal cancer.

**MeSH Keywords:** **Chemokine CX3CL1 • Chemokines • Esophageal Neoplasms**

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 2042  4  —  25



## Background

Esophageal cancer (EC), a cancer with a high incidence rate in the world, can be divided into esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (AC) according to the pathological characteristics [1,2]. Currently, the mortality rate of EC has been reduced prominently, but the survival rate of the patients is still far from satisfying. The main reason is that EC is prone to invasion and metastasis in the early stage, submucosal invasion of the tumor cells occurs easily in the early stage, and the infiltration, invasion, and distant metastasis of the tumor cells are realized through the widespread mucosal and submucosal lymph network and vascular network, so the patients lose the opportunity to receive radical resections [3]. Therefore, investigating the metastasis and invasion mechanisms of EC is an important direction for the treatment of the disease.

Chemokines are small molecules capable of mediating the directed motion of cells, which are composed of 4 subfamilies (C, C-C, C-X-C, and C-X3-C) according to different structures [4]. There is a bi-directional relationship between chemokines and tumors: on the one hand, chemokines can activate the immune responses in the body to kill the tumor by regulating the infiltration of leukocytes to the tumor [5–7]. On the other hand, they can promote the angiogenesis in tumor tissues, accelerate the proliferation of tumor cells and drive the tumor cells to invade into the basement membrane, thus helping the growth and metastasis of the tumor [8–10]. In order to explore the correlations of the chemokines (CX3CL, CXCL-11, CXCL-12, CCL3, CCL4, CCL20) with EC metastasis, pathological type and prognosis judgement, the expression levels of the serum chemokines were detected in this study.

## Material and Methods

### Study participants

A total of 50 patients initially diagnosed with EC (finally confirmed through pathology) in The 1st Affiliated Hospital of Bengbu Medical College from January 2015 to October 2017 were enrolled into EC group, and the serum specimens were collected before operation. None of the patients received treatments such as radiotherapy, chemotherapy, or biological therapy. There were 37 males and 13 females, aged 30 to 73 years old, with a median age of 56 years old. There were 17 cases of non-metastatic EC, 33 cases of metastatic EC, 36 cases of ESCC, and 14 cases of AC in the EC group. According to the cancer staging of EC patients included in our study, 4 patients were in stage I, 10 patients were in stage II, 25 patients were in stage III, and 11 patients were in stage IV. Meanwhile, the serum specimens of 50 normal people in the physical

examination center were collected. There were 32 men and 18 women, aged 39 to 75 years old, with a median age of 59 years old. Signed written informed consent was obtained from all the participants before the study. This study was approved by the Ethics Committee of the 1st Affiliated Hospital of Bengbu Medical College.

### Collection of blood samples

A total of 2 mL fasting peripheral blood was drawn from each group of patients, which was centrifuged at 2500 rpm; 7 minutes later, the supernatant was sub-packed and frozen in an ultra-low-temperature refrigerator at  $-80^{\circ}\text{C}$ .

### Measurements of chemokines levels

By virtue of the microsphere suspension array, liquid chip for short, fluorescence-encoded microspheres covalently cross-linked with monoclonal antibodies were combined with target molecules to be detected, and then fluorescein-labeled antibody was added. Finally, the concentration of molecules to be detected was determined by identifying a single microsphere via laser scanning of fluorescence codes and measuring the fluorescence intensity. In this study, the Luminex-200™ instrument with xPONENT®3.0 software was utilized to detect the samples to be detected and capture the images.

### Statistical analysis

All statistics were performed using Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA). It was tested that each chemokine did not conform to the normal distribution and was expressed as the median (M) and interquartile range (IQR). The Mann-Whitney U test was used for comparison between groups, and Spearman correlation analysis was performed to test the correlation. The test level was  $\alpha=0.05$ .  $P<0.05$  suggested that the difference was statistically significant.

## Results

The median expression levels [interquartile ranges (IQRs)] of CX3CL1, CXCL-12, and CCL20 were 116.74 (118.35), 1.28 (1.04), and 10.14 (8.93), respectively, in EC patients, which were 62.54 (75.02), 7.62 (8.15), and 9.02 (7.57), respectively, in normal control group. The differences were statistically significant between the 2 groups ( $Z=-4.121$ ,  $P<0.001$ ,  $Z=-1.075$ ,  $P<0.001$ ,  $Z=-3.146$ ,  $P=0.003$ , Table 1). The differences in the expressions of serum CXCL-11, CCL3, and CCL4 between EC group and normal control group were not statistically significant ( $P>0.05$ , Table 1).

**Table 1.** Expressions of serum chemokines in EC group and normal control group [M(QR), pg/mL].

Chemokine	Control group (n=50)	EC group (n=50)	Z	P
CX3CL1	62.54 (75.02)	116.74 (118.35)	-4.121	<0.001
CXCL-11	13.98 (14.06)	17.15 (17.02)	-2.032	0.159
CXCL-12	7.62 (8.15)	12.83 (10.49)	-1.075	<0.001
CCL3	14.59 (8.83)	14.72 (13.96)	-0.354	0.628
CCL4	13.27 (13.64)	14.36 (10.58)	-1.693	0.315
CCL20	9.02 (7.57)	10.14 (8.93)	-3.146	0.003

$P < 0.05$  suggested statistical significance.

**Table 2.** Expressions of serum chemokines in non-metastatic EC group and metastatic EC group [M(QR), pg/mL].

Chemokine	Non-metastatic EC group (n=17)	Metastatic EC group (n=33)	Z	P
CX3CL1	103.25 (112.34)	146.87 (153.52)	-2.147	0.169
CXCL-11	15.87 (12.06)	19.98 (20.15)	-1.359	0.204
CXCL-12	13.14 (10.73)	12.43 (10.16)	-0.281	0.783
CCL3	14.86 (17.85)	14.22 (13.28)	-0.725	0.626
CCL4	13.02 (12.97)	15.87 (10.13)	-1.236	0.317
CCL20	9.93 (6.72)	11.90 (10.31)	-0.832	0.150

$P < 0.05$  suggested statistical significance.

**Table 3.** Expressions of serum chemokines in ESCC group and AC group [M(QR), pg/mL].

Chemokine	ESCC Group (n=36)	AC Group (n=14)	Z	P
CX3CL1	108.24 (107.38)	129.23 (125.17)	-3.074	0.465
CXCL-11	23.25 (21.61)	15.87 (14.32)	-2.135	0.226
CXCL-12	15.06 (11.35)	11.28 (9.83)	-0.973	0.149
CCL3	12.09 (13.02)	15.84 (14.06)	-1.428	0.247
CCL4	13.37 (9.29)	18.45 (11.94)	-2.039	0.031
CCL20	10.08 (8.14)	10.83 (9.95)	-0.216	0.924

$P < 0.05$  suggested statistical significance.

There were no statistically significant differences in the expressions of the 6 chemokines between metastatic EC group and non-metastatic EC group ( $P > 0.05$ , Table 2). However, the expressions of 4 chemokines, except CXCL-12 and CCL3, were increased in metastatic EC group compared with those in non-metastatic EC group ( $P > 0.05$ , Table 2).

AC group had higher expression of serum CCL4 than ESCC group, with a statistically significant difference ( $Z = -2.039$ ,  $P = 0.031$ , Table 3), while no statistically significant differences

in the expressions of the remaining 5 chemokines were detected between the 2 groups ( $P > 0.05$ , Table 3).

The correlations among chemokines between EC group and normal control group are shown in Table 4. CCL20 was positively correlated with CX3CL in both groups ( $r = 0.649$ ,  $P < 0.001$ ,  $r = 0.758$ ,  $P < 0.001$ ). Moreover, CXCL-12 had a positive correlation with CCL3 and CCL20 in normal control group ( $r = 0.531$ ,  $P = 0.039$ ,  $r = 0.515$ ,  $P = 0.025$ ).

**Table 4.** Correlation analysis of chemokines between EC group and control group [r (p)].

Control group	EC group					
	CX3CL1	CXCL-11	CXCL-12	CCL3	CCL4	CCL20
CX3CL1	1.000	0.215 (0.307)	0.337 (0.074)	0.349 (0.422)	0.214 (0.395)	0.758 (0.000)**
CXCL-11	0.254 (0.173)	1.000	0.272 (0.236)	0.258 (0.541)	0.153 (0.385)	0.302 (0.243)
CXCL-12	-0.023 (0.821)	0.314 (0.062)	1.000	0.157 (0.328)	0.049 (0.607)	0.215 (0.434)
CCL3	0.295 (0.312)	-0.068 (0.457)	0.531 (0.039)*	1.000	0.308 (0.642)	0.289 (0.275)
CCL4	0.076 (0.641)	0.042 (0.758)	0.046 (0.863)	0.273 (0.467)	1.000	-0.024 (0.912)
CCL20	0.649 (0.000)**	0.326 (0.218)	0.515 (0.025)*	0.428 (0.087)	0.274 (0.536)	1.000

\*\* The correlation is significant when the confidence level (both sides) is 0.01; \* When the confidence level (both sides) is 0.05, the correlation is significant.

## Discussion

In the early 20th century, Muller first proposed that some chemokine receptors are highly expressed on the surface of tumor cells, and the corresponding chemokine ligands are highly expressed by some tissues or organs at the same time. Therefore, the tumor cells can specifically metastasize toward these organs by binding the chemokine receptors to chemokine ligands together [5–10]. The effects of the same chemokine on varying types of tumor, or those of different chemokines on the same tumor, may be different.

As the only member of the CX3C family, CX3CL1 can increase the adhesion between tumor cells and supply the immune cells [T cells and natural killer (NK) cells] to local tumor, thus reducing infiltration and metastasis. Meanwhile, CX3CL1 is able to enhance the adhesion between tumor cells and vascular endothelial cells, induce tumor vascularization and mediate the infiltration and metastasis of tumor [11]. Park et al. [12] reported that the CX3CL1 expression in breast cancer cells can strengthen the chemotaxis on T cells, NK cells and dendritic cells (DCs), so it can serve as a prognostic marker and novel therapeutic target of breast cancer. In colon cancer and gastric AC, the CX3CL1 level is closely associated with the infiltrating density of lymphocytes and good prognosis of tumor tissues, which can be regarded as an index for judging the risk of tumor recurrence. The introduction of lung cancer cells with stably expressed CX3CL1 into mice can remarkably repress the tumor growth, so CX3CL1 can be taken as a potential therapeutic target of lung cancer. In this study, the content of serum CX3CL1 was increased in EC patients ( $P < 0.05$ ), which is consistent with that in literature reports. However, whether the serum CX3CL1 level can directly reflect the CX3CL1 content in tissues still remains unclear and needs further investigation.

CXCL-12 is capable of coupling to its specific receptor (CXCR4), thereby conveying information and promoting the growth,

attachment, and migration of leukocytes [13]. Several scholars have pointed out that the expressions of CXCL-12 and its receptors in EC tissues are prominently correlated with tumor invasion, neovascularization, lymph node metastasis and patients' prognosis. In previous study, the expression of CXCL-12 level declines in the case of efficacious treatment and rises obviously in the case of disease progression in EC patients [14]. The serum CXCL-12 is also related to lymph node metastasis and distant metastasis, and the correlation with the former is stronger. Moreover, it possesses fairly high sensitivity and specificity in predicting the metastasis of EC. In this study, the serum CXCL-12 level in EC patients was higher than that in normal control group ( $P < 0.05$ ), consistent with the results reported in previous literature [15]. Nevertheless, it is revealed in studies that ovarian cancer cell lines with stably expressed CXCL-12 cannot form tumors, and *in vivo* injection of CXCL-12 antibody can reverse the tumor phenotype and accelerate the tumor growth, suggesting that CXCL-12 may play different roles in various types of tumors [16,17].

The highly expressed CCL20 in tumor tissues can decrease the immune killing effect in the body on tumors and promote tumor growth by virtue of increasing DC accumulation in tumors and inhibiting DC maturation [18]. It has close correlations with the proliferation of multiple types of tumors and the specific metastasis of tissues or organs. Kirshberg et al. [19] reported that CCL20 is able to promote the proliferation of non-small-cell lung cancer (NSCLC), which can serve as a potential therapeutic target of the disease. Previous studies showed that the serum CCL20 level is elevated notably in patients with nasopharyngeal cancer and colorectal cancer, which is associated with the low overall survival rate, local recurrence, and liver metastasis of nasopharyngeal cancer as well as the low overall survival rate and liver metastasis of colorectal cancer [20,21]. In our current study, we found that the serum CCL20 level in EC patients was increased remarkably, which is a finding in line with the aforementioned findings. In addition, in our study,

the serum CCL20 level in the metastatic EC group was higher than that in the non-metastatic EC group ( $P>0.05$ ), implying that CCL20 is possibly associated with EC metastasis.

In the early stage of tumor, CCL3 and CCL4 tend to promote the migration and aggregation of DCs to tumor region and local lymph node and attract the local tumor invasion of active CTL, so it can be used as a potential method for treating tumors [22]. However, such chemotaxis is decreased evidently in the late stage of tumors, and the antitumor immunity is also weakened, so the infiltration, development and even metastasis of the tumors occur easily. CXCL-11 can recruit effector cells, repress angiogenesis, and promote and enhance lymphocyte infiltration in tumor tissues, which may be one of the mechanisms for inhibiting the tumors [23]. Some scholars have discovered that the level of CXCL-11 and CXCL-4 are increased notably in the serum of NSCLC patients. The combined detection of CXCL-11 and CXCL-4 can improve the positive rate of NSCLC diagnosis [24,25]. In our study, the expressions of serum CCL3 and CXCL-11 in the EC group were slightly higher than those in the normal control group ( $P>0.05$ ), suggesting that the patients may have elevated expressions of chemokines dominated by anti-tumor effects to some extent, while the increase cannot repress the growth and metastasis of the tumors.

No statistically significant differences in the expressions of serum chemokines were found between metastatic EC group and non-metastatic EC group in this study ( $P>0.05$ ), but only CXCL-12 and CCL3 expressions were increased in metastatic EC group compared with those in non-metastatic EC group.

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The possible reason is that a small number of EC cases were collected in this study, and further studies need to be conducted with larger sample sizes. It was shown in present study that the serum CCL4 expression in AC group was remarkably higher than that in ESCC group, implying that the expression levels of serum chemokines in tumor patients may be different due to the differences in the pathological type of tumors, and different types of chemokines may affect the tumor growth and metastasis in various ways. CCL20 was positively correlated with CX3CL1 in both groups, and CXCL-12 had a positive correlation with CCL3 and CCL20 in normal control group, indicating that chemokines are characterized by overlapping and synergy, and the combined detection of multiple chemokines can achieve comprehensive and objective evaluation of diseases.

In conclusion, CX3CL1, CXCL-12, CCL20, and other chemokines may be applied to assess the conditions of EC and predict the recurrence risk and blocking or eliminating these highly expressed chemokines may prevent the metastasis of EC, providing new approaches for treatment.

## Conclusions

Expression levels of the serum CX3CL1, CXCL-12, and CCL20 are significantly increased in EC patients, which may be applied to assess the occurrence, development and metastasis of EC.

## Conflict of interest

None.

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