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ORIGINAL ARTICLE

Plasma YKL-40: a Potential Biomarker for Tumor Invasion in Esophageal Cancer

Rongfu Huang [1, *], Jianfeng Yao [4, *], Xin Ding [3, *], Jiangyi Liu [6], Chunmei Fan [1], Hongbin Duan [5], Huiming Ye [2]

** These authors contributed equally to the work*
[1] Department of Clinical Laboratory, The Second Affiliated Hospital, Fujian Medical University, Quanzhou, China
[2] Department of Clinical Laboratory, Women and Children's Hospital, School of Medicine, Xiamen University and Department of Experimental Medicine, School of Public Health, Xiamen University, Xiamen China
[3] Department of Pathology, Zhongshan Hospital, School of Medicine, Xiamen University, Xiamen, China
[4] Reproductive Medicine Centre, Quanzhou Maternity and Child Health Care Hospital, Quanzhou, China
[5] Department of Thoracic Surgery, Zhongshan Hospital, School of Medicine, Xiamen University, Xiamen, China
[6] Quanzhou Municipal Center for Disease Control and Prevention, Quanzhou, China

SUMMARY

Background: **YKL-40, a chitinase-like glycoprotein has been identified as a candidate tumor marker. The current study evaluated the clinical significance of plasma YKL-40 in esophageal cancer patients.**
Methods: **We enrolled 127 esophageal cancer patients, 29 healthy controls. Plasma YKL-40 levels were measured through enzyme linked immunosorbent assay. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic efficiency of plasma YKL-40 in esophageal cancer patients. The correlations between plasma YKL-40 and clinicopathological characteristics of esophageal were analyzed.**
Results: **Plasma YKL-40 levels were significantly higher in patients with lymph node metastasis than those that were non-metastatic (p = 0.005). Patients with tumor thrombus formation presented with significantly higher YKL-40 levels than those without thrombus formation (160.3 vs. 74.7 ng/mL, p = 0.012). YKL-40 levels in patients with advanced stage (III and IV) were significantly higher than those in the early stages (I and II, p = 0.016). ROC curve analysis showed that the area under curve was 0.909, and the best diagnostic threshold of YKL-40 for esophageal cancer was 80.6 ng/mL with 68.9% sensitivity and 96.6% specificity.**
Conclusions: **This study indicated that YKL-40 may be a biomarker for esophageal cancer and potential biomarker for identification of invasive esophageal cancer.**
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Correspondence:
Huiming Ye, PhD
Department of Clinical Laboratory
Women and Children's Hospital
School of Medicine
Xiamen University and
Department of Experimental Medicine
School of Public Health
Xiamen University
Xiamen
China
Phone: +86 5922662071
Email: yehuiming@xmu.edu.cn

Prof. Hongbin Duan
Department of Thoracic Surgery
Zhongshan Hospital
School of Medicine
Xiamen University
Xiamen
China
Email: dhb3100@sina.com

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KEY WORDS

YKL-40, esophageal cancer, tumor invasion, lymph node metastasis, thrombus formation

LIST OF ABBREVIATIONS

ROC - receiver operating characteristic curve
CT - computed tomography
PET/CT - positron emission tomography/computed tomography
TAA - tumor-associated antigen
CEA - carcinoembryonic antigen
CYFRA21-1 - cytokeratin 19 fragment
CA19-9 - carbohydrate antigen 19-9

INTRODUCTION

The global annual incidence of newly diagnosed esophageal cancer is about 450,000. The morbidity and mortality of esophageal cancer rank eighth and fifth among all tumors in developing countries, respectively, much higher than in under-developed countries [1,2]. The incidence of esophageal cancer among males is much higher than among females, and occurrences are much higher in rural areas than in cities [3,4].

At present, the diagnostic tools used for the identification of esophageal cancer are mainly based on endoscopic methods such as barium meal, computed tomography positron emission tomography/computed tomography (CT, PET/CT) esophageal ultrasound and gastroscopy examination [5]. In terms of laboratory examinations, blood markers of esophageal cancer include tumor-associated antigen (TAA) antibodies, such as anti-SURF1, HOOK2, LOC146223, AGENCOURT_7565913, NY-ESO-1 antibodies, etc. One of these, anti-p53 antibody against a variant P53 protein in esophageal squamous cell carcinoma, was found to have a high specificity (98.3%), but its sensitivity was only 26.7% [6]. Carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA21-1), squamous cell carcinoma antigen (SCC-Ag), and carbohydrate antigen 19-9 (CA19-9) also show levels of sensitivity and specificity in the diagnosis of esophageal cancer, and the combination of multiple indicators can improve diagnostic efficiency [7,8]. However, no blood markers with excellent clinical application value that can be used in the diagnosis and treatment evaluation of esophageal cancer have been identified to date.

YKL-40 (chitinase protein-40, or Hcgp-39), also known as chitinase 3-like protein 1 (CHI3L1), is a glycoprotein belonging to one of the members of the mammalian chitinase-like protein family [9]. Studies of breast cancer, gastric cancer, colorectal cancer and gliomas [10-15] have shown that YKL-40 levels can reflect tumor growth and metastasis and can also be used as a prognostic indicator of cancer with a certain level of treatment evaluation value. Recently, Zheng et al. [16] reported that YKL-40 as a marker is superior to CEA, CYFRA21-1, and SCCA in the diagnosis of esophageal squamous cell carcinoma and the combination of YKL-40 and SCCA has better diagnostic efficiency for esophageal squamous cell carcinoma. However, the role of YKL-40 in the development of esophageal cancer and whether it can be used to predict treatment efficacy and prognosis remains unclear.

MATERIALS AND METHODS

Study population

From September 2012 to January 2015, we included 127 pathologically confirmed esophageal cancer patients at Zhongshan Hospital affiliated to Xiamen University in the study, with 29 healthy individuals as controls. Complete clinical and pathological records were recorded. None of the patients presented with any endocrine diseases, osteoarthrosis, or other tumor histories. Patients with newly diagnosed esophageal cancer were subjected to surgery, chemotherapy, radiotherapy, or other treatments. Ethical approval for research related to human use was received and is compliant with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, this study was approved by the ethics committee of Zhongshan Hospital affiliated to Xiamen University. All patients signed written informed consent.

The esophageal cancer group was comprised of 127 patients, of which 65 were newly diagnosed (Table 1), 48 were post-operative, and 14 cases presented with recurrence and metastasis. The healthy individual group was comprised of 29 cases, 15 males and 14 females, with a median age of 40 years old (range: 21 - 73). There was no significant difference in age between the two groups ($p > 0.05$).

According to the clinical stage definition of esophageal cancer in China (Surgery, the 7th Edition, People's Medical Publishing House, Chinese), we set 3 cm and 5 cm as a boundary to identify the tumor size. TNM staging $T_1N_0M_0$, $T_2N_0M_0$, $T_3N_0M_0$ to $T_3N_1M_0$, and $T_4N_xM_0$ to $T_xN_xM_1$ were set as stage I, stage II stage III, and stage IV, respectively, in China.

Sample preparation

Venous blood (4 mL) was collected in the morning from fasting esophageal cancer and control subjects into EDTA-K3 anticoagulant-coated vacuum blood collection tubes. The samples were centrifuged at 1,680 g for 10 minutes at room temperature and the plasma specimens were stored at -80°C for subsequent YKL-40 testing.

Detection of plasma YKL-40

YKL-40 protein enzyme linked immunosorbent assay (ELISA) detection kits were purchased from Quidel Corporation (USA). This commercial two-site, sandwich-type ELISA used streptavidin-coated microplate wells, a biotinylated-Fab monoclonal mouse antibody against human YKL-40 (capture antibody), and an alkaline phosphatase-labeled polyclonal rabbit antibody against human YKL-40 (detection antibody). Bound enzyme activity was detected using p-nitrophenyl phos-

Table 1. Demographics and clinicopathological characteristics of newly diagnosed esophageal cancer patients [*].

	No. of patients	%
Total	**65**	
Gender		
Male	**49**	**75.3**
Female	**16**	**24.7**
Age (years)		
Median	**59**	
Range	**39 - 84**	
Tumor size		
Large	**10**	**15.4**
Medium	**34**	**52.3**
Small	**21**	**32.3**
Clinical stage		
I	**4**	**6.1**
II	**18**	**27.7**
III	**25**	**38.5**
IV	**6**	**9.2**
NA	**12**	**18.5**
Tumor type		
Medulla	**11**	**26.8**
Ulcerative	**20**	**48.8**
Constrictive	**2**	**4.9**
Fungiform	**8**	**19.5**
Cell differentiation		
High	**14**	**25**
Moderate	**35**	**62.5**
Mild	**3**	**5.4**
Adenocarcinoma	**4**	**7.1**
Thrombus formation		
Yes	**17**	**37.8**
No	**28**	**62.2**
Lymph node metastasis		
No	**17**	**37.8**
Proximal	**4**	**8.9**
Distal	**24**	**53.3**

*** Only 41 esophageal cancer patients had tumor type records, 56 patients with newly diagnosed esophageal cancer had cell differentiation records, and 45 patients had thrombus formation and lymph-node metastasis records.**

phate as the substrate. The detection limit of the ELISA was 20 ng/mL and the intra-assay coefficient of variation was < 3.6%. The short-term inter-assay coefficient of variation (over an 11-day period) was < 3.7%, and the long-term inter-assay coefficient of variation (over a 5-year period) was < 8.6%. Notably, plasma bilirubin levels below 300 mg/L, hemoglobin levels of less than 5 g/L, and triglycerides less than 30 g/L have been found not to interfere with the assay [12]. Assays were performed according to the product manual, and samples were examined with a Bio-Rad 550 microplate reader (USA).

Statistical analysis

The original data was recorded in Excel and then processed by SPSS software (version 17.0). In the current study, the concentration of plasma YKL-40 did not meet the normal distribution, so the data were presented using the median values unless stated otherwise. Kruskal-Wallis H test was used to compare values among multiple groups, and Nemenyi Rank Sum test was used to compare values between two groups. The graphics were obtained by Microsoft Office Excel 2003 software. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Comparison of plasma YKL-40 levels in esophageal cancer patients and healthy controls

The plasma YKL-40 concentrations recorded in patients with newly diagnosed esophageal cancer were significantly higher than those in healthy controls (121.0 vs. 33.6 ng/mL, $p < 0.001$, Figure 1).

Plasma YKL-40 differences in patients with esophageal cancer in terms of age and gender

As shown in Table 2, there were no significant differences in plasma YKL-40 levels in patients with esophageal cancer between genders ($p > 0.05$). When we divided the patients, according to age, into three groups, less than 50 years old, 50 - 70 years old, and > 70 years, there were significant differences in the plasma YKL-40 levels among the three groups ($p < 0.001$).

Relationship between plasma YKL-40 and clinicopathological characteristics in patients with esophageal cancer

In order to investigate the relationship between plasma YKL-40 levels and clinicopathological characteristics in patients with esophageal cancer, we analyzed the relationship between YKL-40 and tumor size, site, pathological type, cell histopathological grade, thrombus formation, lymph node metastasis, and clinical stage. The results are shown in Table 3.

There were 54 patients with newly diagnosed esophageal cancer that had tumor size records. According to the clinical stage definition of esophageal cancer in China, we set 3 cm and 5 cm as a boundary and divided them into three groups: small (< 3 cm diameter), medium (3 - 5 cm), and large (> 5 cm) tumor groups. There were significant differences in plasma YKL-40 levels among the three groups ($p < 0.05$), and YKL-40 levels

Table 2. YKL-40 levels (median) in patients with esophageal cancer in terms of gender and age differences.

Group	N	YKL-40 (ng/mL)	p-value
Gender			
Male	49	131.5 (28.5 - 300.0)	0.327
Female	16	136.7 (61.3 - 283.9)	
Age			
≤ 50	7	151.7 (52.6 - 300.0)	< 0.001 *
51 - 70	46	116.6 (28.5 - 300.0)	
> 70	12	135.9 (76.7 - 282.1)	

Table 3. Relationship between clinicopathological features and plasma YKL-40 levels (median) in patients with esophageal cancer.

Clinical pathological features	Classification	N	YKL-40 (ng/mL)	p-value
Tumor size *	Large	5	136.8 (39.3 - 146.9)	0.039
	Medium	28	95.4 (28.5 - 300.0)	
	Small	21	140.8 (38.5 - 300.0)	
Site	Upper	10	155.7 (28.5 - 282.1)	0.017
	Middle	34	92.8 (38.5 - 300.0)	
	Lower	21	161.2 (39.9 - 300.0)	
Tumor type	Medulla	11	69.2 (28.5 - 178.7)	0.204
	Ulcerative	20	148.9 (39.9 - 300.0)	
	Constrictive	2	130.0 (66.9 - 192.9)	
	Fungiform	8	131.9 (44.4 - 274.9)	
Cell differentiation **	Highly	14	130.9 (39.3 - 274.9)	0.064
	Moderately	35	88.4 (28.5 - 300.0)	
	Mildly	3	102.0 (39.9 - 142.8)	
	Adenocarcinoma	4	206.7 (79.3 - 283.9)	
Thrombus formation	Yes	17	160.3 (44.4 - 282.1)	0.012
	No	28	74.7 (28.5 - 300.0)	
Lymph node metastasis	No	17	76.7 (28.5 - 274.5)	0.005
	Proximal	4	95.2 (66.5 - 265.9)	
	Distal	24	135.5 (38.5 - 300.0)	
Clinical stage ***	I	4	68.0 (39.3 - 220.2)	
	II	18	71.6 (28.5 - 274.9)	
	III	25	146.9 (38.5 - 300.0)	0.081
	IV	6	111.0 (44.4 - 277.1)	
	NA	12	94.5 (44.1 - 300.0)	

*** Tumor size: large: diameter greater than 5 cm; medium: diameter between 3 cm to 5 cm; small: diameter less than 3 cm.**
**** Tumor cell pathological grading: Among 56 cases of newly diagnosed esophageal cancer, there were only 4 cases of adenocarcinoma, and the rest were squamous cell carcinoma, which was classified according to the degree of cell differentiation as highly, moderately, and mildly differentiated types.**
***** The case numbers from clinical stage I to IV were 4, 18, 25, and 6, respectively, with 12 cases of unknown stage. The p-value quoted is in terms of multi-stage comparison analysis.**

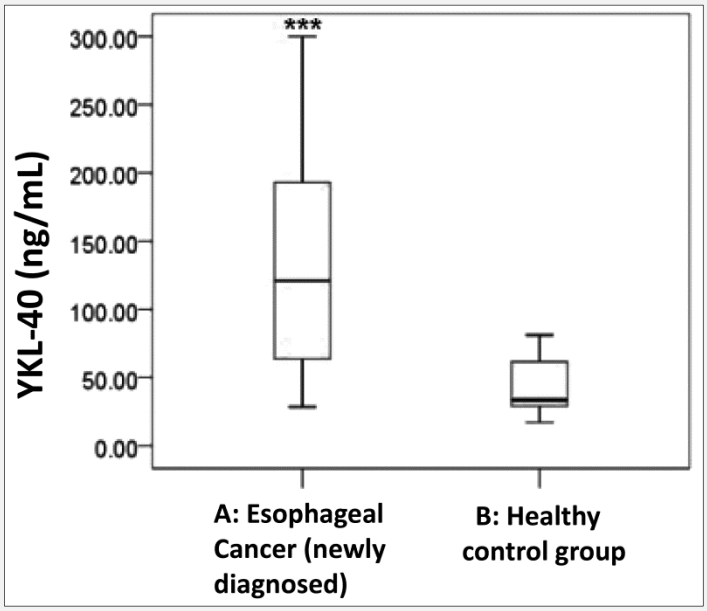

Figure 1. Box-whisker plot for plasma YKL-40 concentrations in the newly diagnosed esophageal cancer group (A) and healthy controls (B).

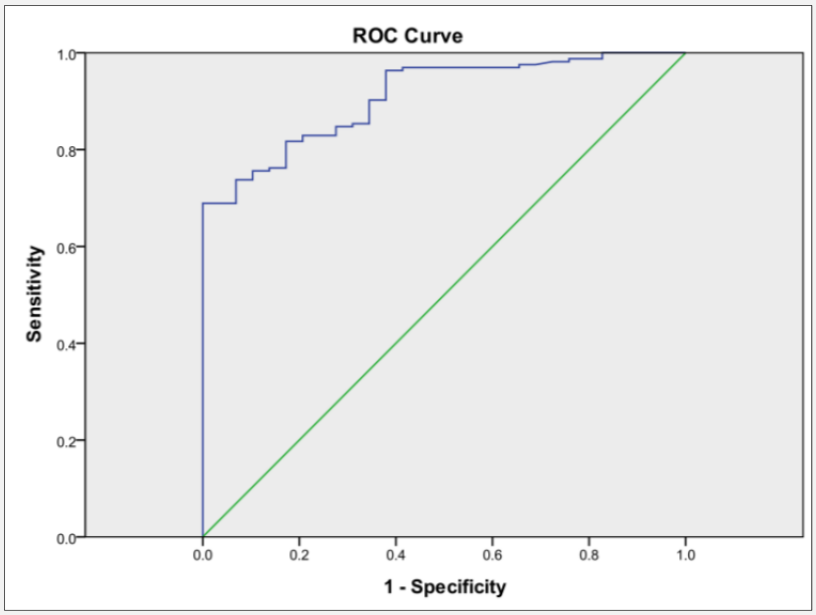

Figure 2. The ROC curve of plasma YKL-40 levels in the diagnosis of esophageal cancer.

were higher in the small and large-sized tumor groups than in the medium-sized tumor group.

When the 65 cases of primary esophageal cancer were sub-classified according to tumor location, YKL-40 levels in patients with esophageal cancer in the middle segment were significantly lower than those in the upper and the lower segments ($p < 0.05$).

Esophageal cancer patients were subdivided into medullary, ulcerative, constrictive, and fungiform types. Among the 41 patients with newly diagnosed esophageal cancer with related records, patients with an ulcerative type exhibited the highest plasma YKL-40 levels, medullary and constrictive in the middle, and the medullary type had the lowest level. However, there were no significant differences amongst the groups ($p > 0.05$).

Esophageal squamous cell carcinoma patients were subdivided into three types by cell differentiation: highly differentiated, moderately differentiated, and poorly differentiated. Among the 56 patients with newly diagnosed esophageal cancer who had cell differentiation records, there were no differences in terms of the plasma YKL-40 levels among the three categories ($p > 0.05$), which indicates that the degree of cell differentiation had no effect on plasma YKL-40 expression in esophageal squamous cell carcinoma.

Among the 45 patients with newly diagnosed esophageal cancer who presented with cancer thrombus formation, the median plasma YKL-40 levels in 17 patients with thrombosis was 160.3 ng/mL, which was significantly higher than that in 28 patients without thrombosis (74.7 ng/mL, $p < 0.05$).

Among the 45 patients with newly diagnosed esophageal cancer who had lymph node metastasis examination, 24 had distal lymph node metastasis, 4 had proximal lymph node metastasis, and 17 had no lymph node metastasis. The expression level of plasma YKL-40 significantly increased along with lymph node metastasis distance. The median level of plasma YKL-40 was 76.7 ng/mL, 95.2 ng/mL, and 135.5 ng/mL in the non-metastasis group, the proximal metastasis group, and the distal metastasis group ($p < 0.05$), respectively.

Among the 65 patients with newly diagnosed esophageal cancer, there were 4 cases with clinical stage I, 18 cases with stage II, 25 cases with stage III, 6 cases with stage IV, and 12 cases with unknown stage. YKL-40 levels in patients in the advanced stages (stage III and IV) were significantly higher than those in stage I and II patients ($p < 0.05$).

The diagnostic efficiency of plasma YKL-40 quantification in esophageal cancer

We evaluated the diagnostic efficiency of YKL-40 quantification for esophageal cancer by using the gold standard (pathological diagnosis) as a reference. The case group included 65 patients with initial esophageal cancer based on the gold standard; the control group was comprised of 29 healthy individuals. Receiver operating characteristic (ROC) curve analysis indicated that YKL-40 cutoff levels of 80.6 ng/mL attained the highest diagnostic efficiency with an area under curve (AUC) of 0.909 (Figure 2). At this threshold, the diagnostic sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 68.90%, 96.60%, 97.83%, 58.33%, and 77.66%, respectively.

Plasma YKL-40 levels in post-operative esophageal cancer patients

The plasma YKL-40 in 48 patients with post-operative esophageal cancer and 14 patients with recurrence and metastasis were both higher than healthy controls (204.1 vs. 33.60 ng/mL, $p < 0.001$ and 166.3 vs. 33.60 ng/mL, $p < 0.001$).

DISCUSSION

In the current study, we found that plasma levels of YKL-40 were significantly higher in the newly diagnosed esophageal cancer group than in the healthy control group, which is consistent with results reported for other types of tumors such as colorectal cancer, ovarian cancer, prostate cancer, endometrial cancer [10,13,15, 17,18], etc. These results indicate that YKL-40 levels might be used as a diagnostic biomarker for esophageal cancer. The results of our current study indicate that YKL-40 may be of value in the diagnosis of esophageal cancer. However, the study population, especially the high-risk population, should be expanded to further investigate the value of YKL-40 in the early diagnosis and screening of esophageal cancer.

YKL-40 levels are age-related, but not gender-related, because YKL-40 is a self-controlled secreted protein and its secretion increases with aging. The level of YKL-40 varies with the tumor size. The YKL-40 levels in large (with tumor diameter greater than 5 cm) and small (with tumor diameter less than 3 cm) groups were significantly higher than in the medium (with tumor diameter 3 - 5 cm) group, suggesting that YKL-40 may play an important role in the early development and late metastasis of esophageal cancer. YKL-40 may play a role in the development of esophageal cancer during the tumor enlargement progress. We speculate that the mechanism may be related to the promotion of cell deterioration, which is likely to create a peripheral environment suitable for the growth and proliferation of tumor cells. YKL-40 levels in patients with distant lymph node metastasis are significantly higher than those with proximal lymph node metastasis and without lymph node metastasis. YKL-40 levels are also higher in patients with tumor thrombus formation than those without. The current study also confirmed that patients with aggressive esophageal cancer exhibit stronger expression of YKL-40, with more YKL-40 being secreted into the peripheral circulation; thus, higher levels of plasma YKL-40 were detected. Interestingly, we also found that YKL-40 levels were much higher in the upper and lower types of esophageal cancer than in the middle type.

Esophageal cancer can occur at different sites and the pathways of invasion and metastasis are different. For example, cancer occurring in the upper part may spread to the neck or the brain, while the lower part may spread downwards to the abdominal cavity. Why YKL-40 levels differ in esophageal cancers occurring at different sites is related to its diffusion pathways and what role it plays in metastasis and spreading are interesting questions that need to be further probed.

Clinical staging of esophageal cancer is based on the degree of invasion of the primary tumor site, lymph node metastasis, distant metastasis, etc. Our study found that plasma YKL-40 expression in patients with advanced esophageal cancer was significantly higher than those at the early stage, which indicates that plasma YKL-40 levels are correlated to the clinical stage of esophageal cancer, and may predict the disease progress and prognosis of esophageal cancer. Tumor cell histopathological grading is based on cell differentiation, nuclear atypia size, and mitotic figures within cells. In general, the higher the grade, the greater the degree of malignancy, the worse the prognosis. In this study, the plasma YKL-40 levels in esophageal adenocarcinoma were found to be significantly higher than in squamous cell carcinoma. However, given the limitation of sample size of our patients with esophageal adenocarcinoma, further research is needed. There were no significant differences among the various subtypes of squamous cell carcinomas.

In the current study, we pooled the esophageal cancer and healthy control groups for ROC curve analysis to evaluate the diagnostic efficiency of YKL-40 as a marker for esophageal cancer. The AUC under the ROC curve was 0.909. The diagnostic threshold was 80.59 ng/mL with 68.90% sensitivity and 96.6% specificity with a concordance rate of 77.66%. The positive likelihood ratio was 20.26, while the negative likelihood ratio was 0.32. The negative predictive value was 58.33%, while the positive predictive value was 97.83%. The ROC curve analysis suggests that YKL-40 can be used as a novel diagnostic marker for esophageal cancer. In the current study, the plasma YKL-40 in patients with post-operative esophageal cancer and recurrence or metastasis were both higher than healthy controls and even higher than the patients with newly diagnosed esophageal cancer. As human YKL-40 could be expressed by neutrophils [11], the higher YKL-40 level in the post-operative patients should be associated with the higher neutrophils counts.

CONCLUSION

In agreement with a previous study [16], our study provided independent evidence to support the clinical significance of elevated plasma YKL-40 protein levels in patients with esophageal cancer, and found that YKL-40 is associated with lymph node metastasis, tumor thrombus formation, clinical stage, and invasiveness of esophageal cancer. Our results indicate that YKL-40 levels correlate with the invasion and metastasis of esophageal cancer but its underlying mechanism still needs to be delineated. As our study was retrospective research, it lacked a validation cohort.

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Declaration of Interest:
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