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Effect of neoadjuvant therapy on serum transforming growth factor-β, squamous cell carcinoma associated antigen, and prognosis in patients with locally advanced esophageal cancer

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ABSTRACT

It was to explore the effect of neoadjuvant therapy (NAT) on serum-related indicators and prognosis of patients with locally advanced esophageal cancer (EC). 400 EC patients were grouped as controls (295 cases, radical EC resection alone) and research group (105 cases, NAT plus radical EC resection). The levels of serum carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), and cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), programmed death-1 (PD-1), PD-2, transforming growth factor-β1 (TGF-β1), and squamous cell carcinoma (SCC) antigen were detected before and after treatment. The follow-up lasted for 3 years. The quality of life (QoL) was evaluated by QLQ-OES24. The recurrence rate, recurrence time, overall survival rate (SR), disease-free SR, and complication rate were compared. Compared with controls, the levels of serum CA19-9, CEA, CYFRA21-1, PD-1, PD-2, TGF-β1, and SCC were decreased, the QoL score was increased 3 years post-treatment, and the recurrence time was prolonged in the research group ($P<0.05$). The R0 resection rate, recurrence rate, 3-year overall SR, and disease-free SR of the two groups were 67.12% vs 85.71%, 21.36% vs 6.67%, 56.27% vs 77.14%, 29.83% vs 45.71%, respectively ($P<0.05$). The complication rates of the two groups were 32.54% and 29.52%, respectively ($P>0.05$). NAT plus radical resection of EC can effectively reduce the level of serum oncology markers in patients with locally advanced EC, reduce the postoperative recurrence rate, improve QoL and SR, and has high safety.

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Introduction

EC is a very common malignant tumor of the digestive tract. The main pathological type is esophageal SCC, and postoperative recurrence and metastasis are the main factors leading to death (1). With the change in people's lifestyles, the incidence of EC has seriously affected the QoL and health of patients. Surgery is the preferred method for the treatment of EC in clinical practice, which is applied for the treatment of early EC, with a high resection rate and high long-term SR of patients (2,3). The typical clinical symptom of EC is dysphagia, but the early symptoms are not obvious. Therefore, EC is difficult to be detected in the early stage, and effective diagnosis and timely treatment can't be achieved. Statistics show that about 80% of EC patients have progressed to a locally advanced stage at the time of diagnosis. The surgical resection rate of such patients is low, and the 5-year SR after surgery is less than 25%, and the recurrence or metastasis is likely to occur within 2 years after surgery (4-6). NAT can markedly reduce tumor volume, reduce tumor burden, and kill micro-metastatic lesions, which has achieved excellent efficacy in the treatment of locally advanced non-small cell lung cancer, breast cancer, colon cancer, and other diseases (7). Studies have confirmed that preoperative NAT for patients with locally advanced EC can obviously improve the resection rate of lesions and the postoperative SR (8,9). However, the clinical effect and postoperative complications of NAT in the preoperative treatment of EC are still controversial.

Tumor markers are commonly adopted for early diagnosis of tumors. At present, there are few tumor markers for EC diagnosis and a lack of specificity. CA19-9, CEA and CYFRA21-1 are common serological markers for EC diagnosis, efficacy evaluation, and prognosis analysis (10). Tumor angiogenesis is the basis of tumor growth and metastasis. Studies have shown that TGF-β1 indicates a high expression trend in the neovascularization of malignant tumor tissues, so it can be applied to reflect the proliferation of tumor cells and the degree of tumor malignancy (11). SCC is widely adopted in the diagnosis of cervical cancer, which is mainly encoded by SCCA1 and SCCA2 genes (12). Dynamic monitoring of the levels of tumor markers provides the possibility to monitor the prognosis of EC patients, and improve or select appropriate treatment methods, improving the SR of patients.

The effect of preoperative NAT on the levels of serum oncology markers and prognosis of patients with locally advanced EC were evaluated. The aim was to provide

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ideas for the formulation and selection of treatment regimens for locally advanced EC.

Materials and Methods

General information

The clinical data of 400 patients with locally advanced EC who were hospitalized in the Department of Thoracic Surgery of Huai'an No.1 People's Hospital from January 2018 to March 2020 were enrolled. The patients who underwent preoperative NAT plus surgery were grouped into a research group (105 cases) and the patients who underwent surgery alone were divided into controls (295 cases). Inclusion criteria: (I) age of 18-70 years old; (II) EC confirmed by cytology or pathology; (III) ECOG score 0-1 and expected survival time more than 6 months; (IV) resectable locally advanced EC assessed by chest and abdominal imaging or endoscopic ultrasonography pretreatment; (V) cT3-4NxM0 according to the AJCC clinical stage, 8th edition, without distant metastasis; (VI) newly diagnosed patients with no history of other systemic malignant tumors; (VII) routine blood test, liver, kidney, and heart function were normal. Exclusion criteria: (I) cervical or abdominal EC; (II) multiple primary cancers or malignant tumors in other parts of the body; (III) underlying immune system diseases; (IV) patients intolerant to chemotherapy/radiotherapy or surgery; (V) women who are pregnant or lactating; (VI) severe respiratory or circulatory system dysfunction; (VII) patients with tumor progression after NAT or unable to undergo surgical treatment; (VIII) patients with cognitive impairment.

Controls had 221 men and 74 women. The average age was (57.3±3.9) years. The mean body mass index (BMI) was (23.0±2.3) kg/m^2. The average tumor diameter was (5.6±1.9) cm. The tumors were in the upper thoracic segment in 77 cases, the middle thoracic segment in 128, and the lower thoracic segment in 90. Pathological types were divided into 223 cases of SCC, 32 cases of adenocarcinoma, and 40 cases of others. There were 79 men and 26 women in the research group, with a mean age of (57.7±3.8) years, BMI of (23.3±2.1) kg/m^2, and tumor diameter of (5.4±1.7) cm. The tumors were in the upper thoracic segment in 27 cases, the middle thoracic segment in 46, and the lower thoracic segment in 32. There were 79 cases of SCC, 12 cases of adenocarcinoma, and 14 cases of others. There was no marked difference in general data between the two groups (P>0.05).

Treatment methods

Controls were treated with radical EC resection. Broad-spectrum antibiotics were given intravenously 1 to 2 days before surgery, and plain and enhanced CT scans of the head, neck, chest, and abdomen were carried out. Blood routine and other examinations were performed to determine the surgical treatment plan. Complete resection of the lesion, lymph node dissection, gastroesophageal anastomosis, and digestive tract reconstruction was carried out. A duodenal feeding tube was indwelling during the operation, and an enteral nutrition solution was instilled 1-day post-operation. After the operation, low-flow oxygen inhalation, electrocardiogram monitoring, aerosol inhalation, gastrointestinal decompression, and intravenous antibiotics were given. A liquid or semi-liquid diet was given for 7 days, and a normal diet was given 30 days following an operation.

The research group completed NAT before surgery, and the treatment plan was as follows: on the first day, intravenous infusion of paclitaxel 135 mg/m^2; 30 mg/m^2 cisplatin was given intravenously from day 1 to day 3. Three weeks was a course of treatment, a total of 3-4 courses. The patients were followed up 4 weeks after treatment and underwent surgery. The radiotherapy regimen before surgery was as follows: conventional fractionation 200 cGy/d, 5 days a week, a total of 20 times, a total dose of 40 Gy; The target volume was divided into the tumor target area and clinical target area.

Detection of serum-related indicators

Fasting venous blood (3 mL) was collected from the patients before and after treatment, and the serum was separated by centrifugation at 3,000 rpm/min for 20 min at room temperature. The levels of serum tumor markers CA19-9, CEA, CYFRA21-1, PD-1 and PD-2, TGF-β1, and SCC were detected by ELISA. The cut-off values of CA19-9, CEA, and CYFRA21-1 were 39 U/ml, 3.4 ng/ml, and 3.3 ng/ml, respectively. Exceeding the cut-off value was considered positive, while the serum oncology marker post-treatment decreased by more than 10% compared with that before treatment, and the index was reduced. ELISA kits were purchased from Beijing Bioss Biotechnology Co., LTD or Beijing Zhongshan Golden Bridge Biotechnology Co., LTD.

Evaluation criteria of therapeutic effect

According to the WHO solid tumor response evaluation criteria, the treatment response was scored as complete remission (CR), partial remission (PR), progressive disease (PD), and stable disease (SD). CR: All lesions disappeared, persisting for at least 4 weeks. PR: a reduction of more than 50% in the maximum diameter of the lesion, persisting for at least 4 weeks. SD: a reduction of 25 to 50% in the maximum diameter of the lesion, persisting for at least 4 weeks. PD: the maximum diameter of the lesion increased by more than 25% or new lesions appeared. Objective response rate (ORR)=(CR+PR)/(CR+PR+SD+PD)×100%. Disease control rate (DCR)=(CR+PR+SD)/(CR+PR+SD+PD)×100%.

Follow-up methods

Patients were followed up by telephone or outpatient examination, and the recurrence rate and recurrence time were recorded. The QLQ-OES24 (13) by EORTC was adopted to evaluate the QoL of patients. The scale included 20 dimensions and the maximum score was 100 points. The 3-year SR and postoperative complications were recorded.

Statistical methods

SPSS 22.0 statistical software was adopted to analyze and process data. Enumeration data were presented as rate (%), and chi-square test to compare the distinction of groups. Measurement data were presented as mean ± standard deviation, and t-test was adopted to compare between groups. The Kaplan-Meier survival curve was drawn to analyze the survival of the two groups, and the Log-Rank test to compare the survival between the two groups. The distinction between groups was considered statistically meaningful when P<0.05.

Results

Clinical effect of NAT for EC

The clinical effects of NAT for 105 cases of EC were analyzed (Table 1). The CR rate was 44.76%, and the PD rate was 10.48%. The ORR and DCR were 77.14% and 89.52%, respectively. R0 resection was achieved in 198 cases (67.12%) in controls and 90 cases (85.71%) in the research group. The R0 resection rate in the research group was higher (P<0.05).

Comparison of serological indicators of patients

The serum levels of CA19-9, CEA, and CYFRA21-1 in the controls and research group post-treatment were lower than before treatment, and those in the research group were lower as against controls (P<0.05) (Figure 1).

The levels of PD-1 and PD-2 in the serum of the patients are illustrated in Figure 2. Relative to before treatment, the serum levels of PD-1 and PD-2 in the controls and research group post-treatment were decreased, and as against controls, the levels of serum PD-1 and PD-2 in the research group were decreased after treatment (P<0.05).

The levels of TGF-β1 and SCC in the serum of the patients are given in Figure 3. The levels of serum TGF-β1 and SCC in the controls and research group following treatment were lower relative to before treatment, and the levels of serum TGF-β1 and SCC in the research group following treatment were lower as against controls (P<0.05).

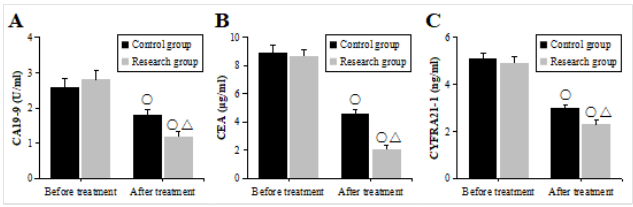

Figure 1. The contrast of serum CA19-9 (A), CEA (B), and CYFRA21-1 (C) levels in patients. Compared with the same group before treatment, °P <0.05; Compared with controls, $^{\Delta}P$ <0.05.

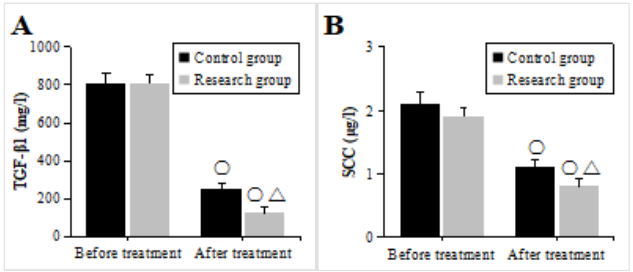

Figure 2. The contrast of serum PD-1 (A) and PD-2 (B) levels in patients.

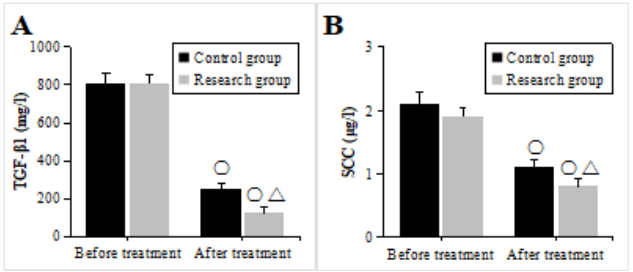

Figure 3. The contrast of serum TGF-β1 (A) and SCC (B) levels in patients.

Table 1. Evaluation of the effect of NAT for EC.

	Case	Proportion %
Sample size	105	
CR	47	44.76
PR	34	32.38
SD	13	12.38
PD	11	10.48
ORR	81	77.14
DCR	94	89.52

Note: CRis complete remission; PRis partial remission; SDis stable disease; PDis progressive disease; ORRis objective response rate; DCRis disease control rate.

Comparison of QoL, recurrence rate, and recurrence time of patients

The QoL scores of the patients and the recurrence rate, and the recurrence time post-treatment are illustrated in Figure 4. Compared with before treatment, the QoL scores of two groups raised following treatment; as against controls, the QoL score of the research group raised following treatment. After treatment, 63 cases (21.36%) relapsed in controls and 7 cases (6.67%) relapsed in the research group. The average recurrence time was (22.5±2.1) months in controls and (28.4±3.0) months in the research group; the recurrence rate was decreased and the recurrence time was prolonged in the research group (P<0.05).

Comparison of 3-year SR

Kaplan-Meier curves for 3-year postoperative survival are given in Figure 5. The 3-year overall SR and disease-free SR of controls were 56.27% and 29.83%, respectively. In the research group, the 3-year overall SR was 77.14%, and the 3-year disease-free SR was 45.71%. The 3-year overall SR and disease-free SR were raised in the research group (P<0.05).

Comparison of complication rates

The differences in the probabilities of various com-

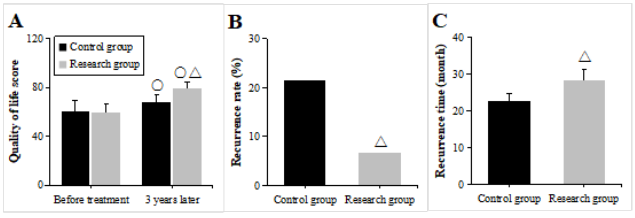

Figure 4. TThe contrast of QoL scores (A), recurrence rate (B), and recurrence time (C) levels.

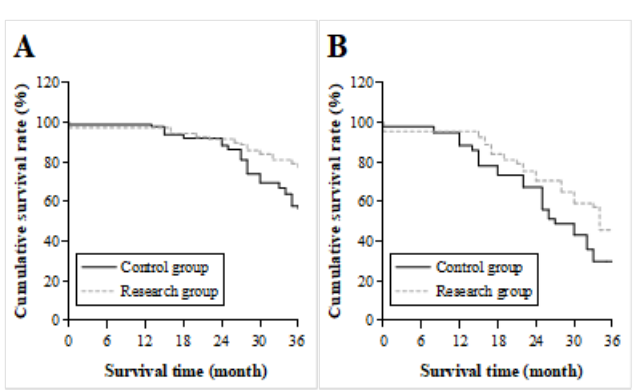

Figure 5. 3-year postoperative overall SR (A) and disease-free SR (B).

plications during treatment and follow-up are illustrated in Figure 6. There were 77 cases (26.10%) of pulmonary infection, 40 cases (13.56%) of anastomotic leakage, 21 cases (7.12%) of arrhythmia, 14 cases (4.75%) of active bleeding in the operation area, 11 cases (3.73%) of intestinal obstruction, and 5 cases (1.69%) of wrapped pleural effusion in controls. The total complication rate was 32.54%. The research group had 25 cases (23.81%) of pulmonary infection, 12 cases (11.43%) of anastomotic leakage, 8 cases (7.62%) of arrhythmia, 4 cases (3.81%) of active bleeding in the operation area, 3 cases (2.86%) of intestinal obstruction, and 2 cases (1.90%) of wrapped pleural effusion. The total complication rate was 29.52%. There was no significant difference in the incidence of complications between the control and research groups (P>0.05).

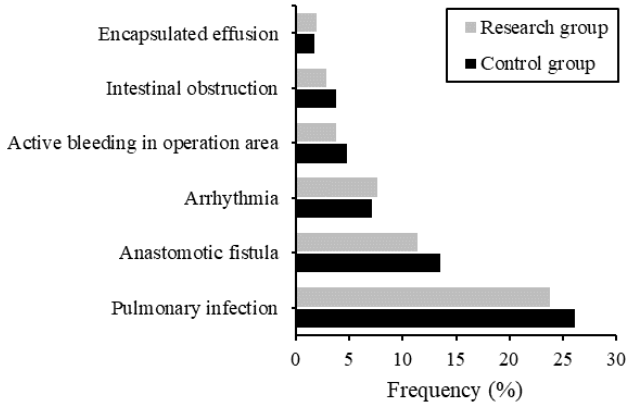

Figure 6. The contrast of postoperative complication rates.

Discussion

EC is a kind of malignant tumor with high incidence, and its incidence has been increasing year by year in recent years. Surgical resection is the preferred treatment for EC, but the long-term prognosis of patients treated with surgery alone is poor (14). In addition, the clinical symptoms of early EC are relatively insidious, and many patients are in the middle and late stages when diagnosed, even have distant metastasis, so they miss the best time for surgical treatment. NAT refers to systemic chemotherapy administered before surgery. The purpose of treatment is to reduce the lesion and kill the metastatic cells as soon as possible, to improve the effectiveness of surgery or radiotherapy (15,16). The effect of preoperative NAT on the therapeutic outcome of patients with locally advanced EC undergoing radical resection was explored, and the ORR was 77.14% and the DCR was 89.52%. Oguma et al. (2019) (17) showed that the R0 resection rate of patients with neoadjuvant chemotherapy combined with surgical resection was clearly higher than patients with surgery alone, and the postoperative local recurrence rate was obviously reduced. It is similar to the results of the present study, which found that the R0 resection rate of EC patients with preoperative NAT plus surgical resection was evidently higher as against patients with surgery alone (85.71% vs 67.12%). Postoperative recurrence and metastasis are important factors leading to the reduced long-term survival of EC patients (18). Eyck et al. (2021) (19) analyzed the impact of NAT on the safety and efficacy of locally advanced EC and found that the overall SR of patients with NAT was clearly higher relative to patients with surgery alone, while the distal recurrence rate was reduced during 10 years of follow-up. It is similar to the finding that patients with EC who underwent preoperative NAT plus surgical resection had lower recurrence rates, longer time to recurrence, and increased overall survival and disease-free survival. Surgical resection of EC alone has poor clinical efficacy for patients with locally advanced EC. However, preoperative NAT intervention can markedly improve the clinical diagnosis of EC patients, and reduce tumor staging, thereby improving the success rate and treatment effect of radical resection of EC (20). In addition, there was no obvious difference in the incidence of complications such as pulmonary infection, anastomotic leakage, arrhythmia, active bleeding in the operation area, and intestinal obstruction between EC patients with surgery alone and those with preoperative NAT. Preoperative NAT does not increase the risk of complications in patients undergoing EC radical resection and is safe.

The dynamic changes of tumor markers can indirectly reflect the therapeutic effect of the tumor. CA19-9 is an oligosaccharide antigen secreted by gastrointestinal tumor cells, which is clearly increased in the serum of patients with gastrointestinal cancer (21). CEA is an acidic glycoprotein of human embryonic antigen-specific determinant, which is mainly secreted by organs such as the stomach, intestine, respiratory tract, and urinary tract (22). CYFRA21-1 is widely present in the cytoplasm of epithelial tumor cells such as EC and is released into tissue fluid or body fluids when cancer cells are dissolved or necrotic (23). Ma et al. (2017) (24) confirmed that serum CEA and CYFRA21-1 could accurately predict the prognosis of NAT patients. It was found that the levels of serum CA19-9, CEA, and CYFRA21-1 in EC patients were markedly decreased following different treatment methods, and those in patients with preoperative NAT plus EC radical resection were lower. By binding to their corresponding ligands, PD-1 and PD-2 can activate the corresponding cell signal transduction pathways, induce the apoptosis of T lymphocytes, and enable cancer cells to escape from immune killing (25). Hayata et al. (2018) (26) proposed that the serum levels of PD-1 and PD-2 in EC patients were obviously raised, and were closely associated with the degree of tumor invasion, lymph node metastasis, and tumor clinical stage. It was found that the serum levels of PD-1 and PD-2 in EC patients were clearly decreased following different treatments, and those in patients with preoperative NAT combined with EC radical resection were lower than in patients with preoperative NAT. Preoperative NAT can markedly inhibit the expression of serum tumor markers and the degree of necrosis factor, to inhibit the immune escape of tumor cells and ultimately improve the therapeutic outcome.

TGF-β1 is a kind of cytokine with a variety of biological activities, which can induce local angiogenesis, generate an extracellular matrix, inhibit immune surveillance, and enhance heterogeneous cell adhesion (27). Lu et al. (2015) (28) pointed out that the serum level of TGF-β1 in EC patients was closely correlated with the incidence of pulmonary complications, and the serum level of TGF-β1 in patients with EC was clearly reduced through neoadjuvant concurrent chemotherapy. Cheng et al. (2014) (29) found that the serum TGF-β1 level of EC patients before neoadjuvant concurrent chemotherapy was related

to pathological response and disease-free SR of patients, which was adopted for predicting the prognosis. The present results suggested that the serum level of TGF-β1 in EC patients was decreased after different treatments and that in patients with preoperative NAT plus EC radical resection was lower. The stability of the TGF-β1 level is conducive to maintaining the balance of the internal environment of the body, with the importance to improve the immune function and delay the progression of the disease (30). NAT plus surgery for the treatment of EC patients can evidently reduce the serum level of TGF-β1, which is very important for improving the immune function of patients.

SCC is a group of tumors associated with glycoproteins isolated from cervical SCC, which can be divided into SCCA1 and SCCA2. SCCA2 is mainly expressed in the cytoplasm of cells, which can inhibit the activity of cathepsin G and participate in proteolysis. SCC is a highly specific tumor marker of SCC, which can be applied clinically in the diagnosis and disease progression monitoring of EC, cervical cancer, lung cancer, and other cancer diseases (31-33). Studies have also confirmed that SCCA2 can inhibit tumor cell apoptosis and accelerate tumor growth by inhibiting TNF-α activity (34). Qiao et al. (2019) (35) found the serum SCC expression in EC patients was related to tumor stage, amount of cancerous tissue resection, and lymph node metastasis. The higher the expression level of SCC, the more serious the disease and the worse the prognosis. It revealed that the serum SCC level of EC patients was clearly reduced through different treatment methods and that of patients with preoperative NAT plus EC radical resection was lower following surgery. Preoperative NAT can improve the patient's condition by shrinking the tumor lesion, reducing the tumor stage, inhibiting the spread of cancer cells, and eliminating metastatic lesions, inhibiting the level of tumor markers such as SCC.

NAT plus radical resection of EC can effectively reduce the levels of serum CA19-9, CEA, CYFRA21-1, PD-1, PD-2, TGF-β1, and SCC in patients with locally advanced EC, without increasing the incidence of complications. Only 3-year follow-up data were included to evaluate the prognosis of patients, and more follow-up data need to be further included to analyze the outcome of preoperative NAT on 5- and 10-year SR of EC patients after surgery and to analyze the influencing factors of long-term prognosis of patients. In conclusion, it can provide help for the implementation of preoperative NAT in EC patients.

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