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Plasma levels of IL-6 and TNF-α in patients with esophageal cancer

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Aim: Cytokines are multifunctional polypeptides synthesized in various cell types. Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are cytokines that have important roles in cancer pathogenesis. In this study, we investigated the plasma levels of IL-6 and TNF-α in patients with esophageal cancer.

Materials and methods: Forty-three esophageal cancer patients (24 females and 19 males) who were newly diagnosed and had not yet undergone operation participated in the study. The patients' average age was 61.03 ± 12.4 years. The control group, on the other hand, comprised 43 healthy individuals (22 males and 21 females) with an average age of 56 ± 11.3. Forty-one (95.3%) of the patients had symptoms of dysphagia. The second leading symptom was weight loss, experienced by 32 (74.4%) of the patients. The cancer of 5 (11.6%) of the patients advanced in stage and had distant metastases (2 involving the lung and 3 involving the liver) at presentation. TNF-α (TNF-α-EASIA Kit, DIAsource) and IL-6 (IL-6-EASIA Kit, DIAsource) were studied using the enzyme-linked immunosorbent assay (ELISA) method.

Results: The measured TNF-α plasma levels of the patients and the control group were 12.35 ± 9.69 and 4.62 ± 3.06 pg/mL, respectively ($P < 0.0001$). The average TNF-α plasma levels of the patients with and without weight loss were 14.95 ± 9.96 and 4.77 ± 3.12 pg/mL, respectively ($P = 0.044$). The average IL-6 plasma level of the esophageal cancer patients was 60.30 ± 53.15 pg/mL, and the average IL-6 plasma level of the control group was 6.00 ± 3.26 pg/mL ($P < 0.0001$). The average IL-6 levels of the patients with and without weight loss were 65.22 ± 43.27 and 12.37 ± 6.80 pg/mL, respectively ($P < 0.0001$). There was no statistically significant difference in the IL-6 and TNF-α plasma levels of the patients with and without distant metastasis.

Conclusion: The results show that IL-6 and TNF-α plasma levels may be utilized as tumor markers for the diagnosis of esophageal cancer. However, to definitely conclude this, we need further extensive studies.

Key words: Esophageal cancer, IL-6, TNF-α

Introduction

Esophageal cancer ranks sixth in prevalence among all cancers worldwide. About 386,000 people die of this cancer each year (1). Esophageal cancer is one of the most rapidly progressive diseases and is very difficult to treat. Regional lymph node involvement or distant organ metastasis may develop rapidly in this disease, with early and rapid spread to the esophageal wall and adventitia. Despite aggressive treatments, detailed 5-year survival is only as high as 20% (2,3).

Cytokines are multifunctional polypeptides synthesized from various cell types of the human body. They have important roles in many processes, such as the development of humoral and cellular immune responses, triggering of the inflammatory response, regulation of hematopoiesis, supervision

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of cellular differentiation and reproduction, and wound healing. Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are cytokines that have important roles in cellular differentiation and growth (4-6).

TNF-α is a multifunctional cytokine that is also named cachexin. It has a homotrimeric structure and is synthesized from various cell types, especially from macrophages, monocytes, fibroblasts, endothelial cells, adiposities, B cells, and some tumor cell types (7).

The increased IL-6 and TNF-α levels have a role in the development of cancer in many cancer types. However, the data are not sufficient to show a possible relation between esophageal cancer and cytokines TNF-α and IL-6. In this study, we investigated the levels of TNF-α and IL-6 in esophageal cancer patients and compared these with the levels of TNF-α and IL-6 in the control group.

Material and methods

Selection of the patients

The participants in this study were 43 patients who were diagnosed with esophageal cancer in 2008 and 2009 (Department of Thoracic Surgery, Medical Faculty Hospital, Atatürk University) but had not yet undergone any operation. Diagnosis was made with esophagoscopy and biopsy. Patients who had been treated either with chemotherapy or radiotherapy were excluded from this study. Neither the patients nor the control group had any chronic systemic disease, such as hypertension or diabetes mellitus. Patients treated with any medication were also excluded. The patients included 24 females and 19 males with an average age of 61.03 ± 12.4 years. The control group, on the other hand, consisted of 21 females and 22 males (healthy persons) with an average age of 56 ± 11.3.

The most common symptom observed in the patients was dysphagia, present in 41 (95.3%) of the patients. The second most common symptom was weight loss, present in 32 (74.4%) of the patients. Meanwhile, 79.1% (32/43) of the patients had squamous cell carcinoma and 20.9% (9/43) had adenocarcinoma. The tumor was located in the proximal third of the esophagus in 2 (4.7%) patients, in the middle third of the esophagus in 21 (48.8%) patients, and in the distal esophagus or cardium in 20 (46.5%) patients. Distant organ metastases were present in 5 of the patients who participated in this study (11.6%; 3 involving the liver and 2 involving the lung).

Collection of blood samples and storage

Venous blood samples (2 mL) were collected from both the patients and controls. The samples were kept in tubes anticoagulated with ethylenediaminetetraacetate (EDTA). Blood samples were centrifuged in 10-18 °C at 2000 rpm for 10 min in an air-conditioned centrifugal machine. The plasma of the blood samples was kept in Eppendorf tubes at –80 °C until the analysis time. The specimens were not dissolved more than once, and the samples obtained had no hemolysis or lipemia. Before the analysis, the samples were kept at room temperature until they dissolved.

TNF-α (TNF-α-EASIA Kit, DIAsource) and IL-6 (IL-6-EASIA Kit, DIAsource) were studied using the enzyme-linked immunosorbent assay (ELISA) method.

Statistical analysis

The statistical analysis of the raw data was done with SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Results were analyzed using the Mann-Whitney test and are given as mean ± standard deviation. $P < 0.05$ shows statistical significance.

Results

The measured average TNF-α plasma levels of the patients and the control group were 12.35 ± 9.69 and 4.62 ± 3.06 pg/mL, respectively. The average TNF-α plasma level of the esophageal cancer patients was statistically significantly higher than that of the control group ($P < 0.0001$).

The average TNF-α plasma levels of the esophageal cancer patients with and without weight loss were 14.95 ± 9.96 and 4.77 ± 3.12 pg/mL, respectively. The average TNF-α plasma level of the patients with weight loss was significantly higher than that of the patients without weight loss ($P = 0.044$) (Figure 1).

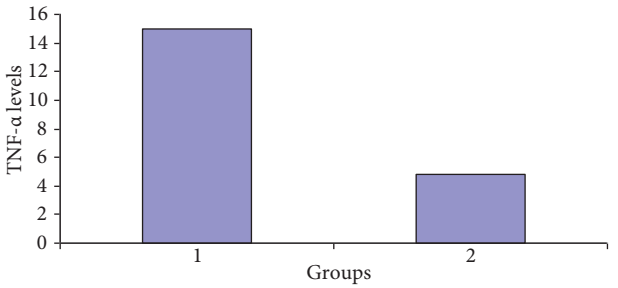

Figure 1. TNF-α levels in patients with weight loss (group 1) and patients without weight loss (group 2).

Although there was a difference in the measured average TNF-α plasma levels of the patients with and without distant organ metastasis (16.44 ± 11.86 and 11.80 ± 9.53 pg/mL, respectively), there was no statistical significance among the groups (P = 0.967) (Figure 2).

The measured average IL-6 plasma levels of the cancer patients and the control group were 60.30 ± 53.15 and 6.00 ± 3.26 pg/mL, respectively. The average IL-6 plasma level of the cancer patients was significantly higher than that of the control group (P < 0.0001).

When we subdivide the cancer patients according to their symptoms, the measured IL-6 plasma levels of the patients with and without weight loss were 65.22 ± 43.27 and 12.37 ± 6.80 pg/mL, respectively. The average IL-6 plasma level of the patients with weight loss was significantly higher than that of the patients without weight loss (P < 0.0001) (Figure 3).

There was no statistically significance difference in the measured average IL-6 plasma levels of the patients with and without distant organ metastasis (62.48 ± 25.16 and 60.01 ± 56.47 pg/mL, respectively) (P = 1.0) (Figure 4).

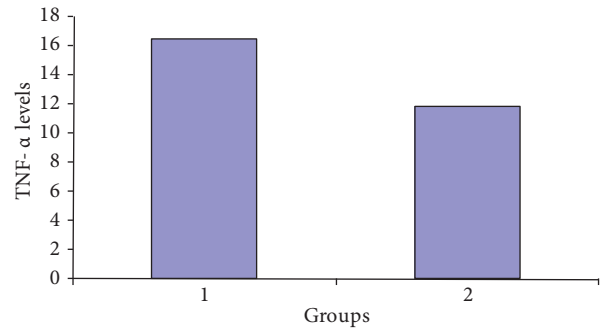

Figure 2. TNF-α levels in patients with metastasis (group 1) and patients without metastasis (group 2).

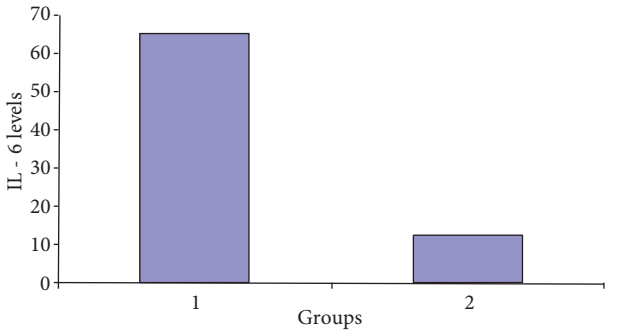

Figure 3. IL-6 levels in patients with weight loss (group 1) and patients without weight loss (group 2).

Discussion

Cytokines play a role in inflammatory response and cancer pathogenesis. Endogenous cytokines are abnormally produced in many kinds of malignancy, either as a server, such as autocrine growth factors, or as an indicator of the immune response to tumors. Cytokine deregulation participates in the initiation and development of malignant processes (8). IL-6 is a hematopoietic cytokine that enables the growth and differentiation of tumor cells (9). IL-6 functions via Janus kinase (JAK) receptors. Many kinds of cancer development can be halted via hypermethylation of the receptors of IL-6. Neutrophil activation is responsible for the increased IL-6 in malignant cases (9).

A recent study that investigated breast cancer and cytokines revealed that high serum levels of cytokines are related to a worse prognosis. It was detected that IL-6 inhibits proliferation via the induction of apoptosis in cancer cells and thus creates DNA fragmentation, which is a characteristic feature of apoptosis in these cells. In that study, it was cited that the IL-6 level of breast cancer patients

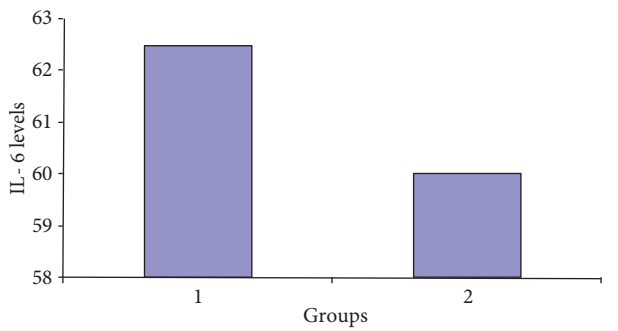

Figure 4. IL-6 levels in patients with metastasis (group 1) and patients without metastasis (group 2).

was 38.3 pg/mL compared with 2.5 pg/mL in the healthy control group (10). In the present study, the IL-6 plasma levels of the esophageal cancer patients and the control group were 60.03 ± 53.15 and 6.00 ± 3.26 pg/mL, respectively. The IL-6 levels of the cancer patients were 10 times higher than those of healthy controls.

The relationship between IL-6 and malignancy is most commonly investigated in relation to ovarian cancer. Van der Zee et al. (11) detected that the origin of IL-6 in ovarian cancer patients was peritoneal mesenchymal cells and cancer cells. They also suggested an inverse relationship between the IL-6 level of cystic fluids and the hemoglobin level. On the other hand, Watson et al. (12), in a study that investigated the treatment of tumor cells with anti-IL-6 antibodies, stated that IL-6 had no effect on tumor growth. Obata et al. (13) reported that IL-6 may enhance tumor growth by affecting the adhesion and migration of the ovarian cancer cells. In another study that investigated the relationship between the IL-6 level and the response of ovarian cancer to chemotherapy, investigators stated that IL-6 levels may have a value in the determination of the progression of ovarian cancer in early phases of the disease and during the chemotherapy period (14).

Metastasis of cancer is a complicated process that involves coordinated cellular effect and the response of both cancer and normal cells. Metastasis has several steps: 1) invasion of the stroma, 2) intravasation of blood vessels, 3) transportation in vasculature, 4) adhesion and implantation to target capillaries, 5) extravasations from blood vessels, and 6) proliferation of secondary tumors (15). IL-6 has a role in steps 1 through 3 of cancer metastasis (15).

Results showed that the IL-6 level has prognostic significance in patients with colorectal cancer that has metastases to the liver and lymph nodes (16). Kemik et al. (9) showed that the serum IL-6 levels of patients with metastatic-stage colon cancer are higher than those of patients without metastasis. De Vita et al. (17), in a study that involved 68 gastrointestinal cancer patients, revealed that the IL-6 level is significantly higher in the patient group than in the control group and that it further increases up to 2 times in patients with metastasis. In the present study, although the IL-6 levels of metastatic patients were higher than those of nonmetastatic patients, the difference was not statistically significant. We included only 5 metastatic patients in the present study. Because of the small number of patients, a statistical analysis was not properly done. We suggest that to get statistically significant results, a higher number of patients should be included in the study.

Weight loss is an important feature in cancer patients. Weight loss is associated with anorexia, decrease in fat and muscle tissue mass, and decrease in survival rate. The etiology of cachexia is multifactorial. Cachexia is commonly accompanied with hypoalbuminemia. Interestingly, the IL-6 level is inversely related to the serum albumin levels of lymphoma patients (18). Besides the preclinical animal studies, other studies showed that IL-6 decreases the synthesis of hepatic albumin production. Other cytokines can also contribute to cachexia. TNF, IL-1, IL-6, interferon-g, and leukemia inhibitory factor are cytokines that lead to weight loss (19).

Some investigators declared that the IL-6 level increases in pancreas cancer patients and that this increase is related to weight loss (20). Falconer et al. (21) also determined that IL-6 is one of the components of cachexia due to increased resting energy expenditure and the presence of acute-phase response in hypermetabolic pancreas cancer patients. In these patients, peripheral blood mononuclear cells spontaneously produce an increased amount of IL-6 (21). In the present study, weight loss was present in 32 (74.4%) of the patients, and the IL-6 levels of the patients with weight loss were higher than those of the patients without weight loss.

In another study that involved 62 preoperative colorectal cancer patients, investigators found that the IL-6 level was nearly 2 times higher in patients than in the control group. In this study, patients had rapid weight loss, sepsis, trauma, and fever, which led to acute-phase response (22).

TNF-α is one of the most commonly studied cytokines and is associated with various cancer types. It enables the destruction of cancer cells and is secreted from various cell types (23). Previous studies showed that IL-6 is a regulator of TNF-α activity. If IL-6 is applied in small amounts, it enhances the cytotoxic effect of TNF-α on human lymphoma cells.

This effect is a result of the overexpression of TNF-α receptors via IL-6 (24).

The exact mechanism of how TNF-α affects the development of cancer is not known yet. It is speculated that it plays a role in the development of cancer via the production of DNA damage and the inhibition of the DNA repair mechanism. In addition to this, TNF may produce tumor invasion in colorectal cancer via c-Src oncogene activation (25). It was previously reported as a macrophage-derived serum factor due to its effect of hemorrhagic necrosis on tumors. The necrotic effect of TNF on tumor cells is due to the destruction of tumor vasculature rather than direct effect on tumor cell (6).

The inflammatory cytokine TNF-α, especially when it is produced from epithelial cells and stromal components, is an important factor in the progression of tumor in animal studies (26). This cytokine is produced from malignant cells of advanced cancers and is considered a bad prognostic factor. Epithelial ovarian cancer is one of the cancer types in which TNF-α production is detected. The TNF-α level is correlated with increased tumor stage and expression of the chemokine receptor CXCR4 in biopsy specimens (27).

Cytokines such as IL-6 and TNF-α have important roles in the prognosis of breast cancer. Moreover, IL-6 inhibits the proliferation of cancer cells by the induction of apoptosis and by DNA fragmentation in cancer cells, which is characteristic of apoptosis (10).

Several studies conducted on soft tissue tumor, melanoma, colorectal, prostate, and gastrointestinal cancer patients showed that IL-6 and TNF-α levels increased from 2-fold to 10-fold compared with the levels in control patients. IL-6 is also a pleiotropic cytokine and is further increased in metastatic patients. These studies emphasized that IL-6 is a precursor factor in cancer prognosis and that TNF-α has clinical importance (16,17,28-31). In the present study, the TNF-α level was 3 times higher in esophageal cancer patients than in the control group. Similar to IL-6, the TNF-α level significantly increased in patients with weight loss compared to patients without weight loss. Furthermore, there was no statistically significant difference in the TNF-α level between the patients with and without metastasis.

A study involving 353 nonsmall-cell lung cancer patients showed that IL-6 is related to bad prognosis in both African Americans and Caucasians. The same study showed that IL-10 and IL-12 in African Americans and TNF-α in Caucasians are related to survival rates (31).

In summary, the IL-6 and TNF-α levels of the esophageal cancer patients in our study were significantly higher than those of the control group. The IL-6 and TNF-α levels were significantly higher in patients with weight loss than in patients without weight loss. This paper is an attempt to contribute to the still inadequate investigation of the effects of cytokines on esophageal cancer. We recommend further extensive investigations on the effects of cytokines on follow-up, prognosis, and tumor growth and particularly their direct effect on esophageal cancer to contribute to the understanding of its pathogenesis.

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