SERUM INTERLEUKIN-12 AND INTERLEUKIN-18 LEVELS IN PATIENTS WITH OESOPHAGEAL SQUAMOUS CELL CARCINOMA

D. Diakowska*, K. Markocka-Mączka, K. Grabowski, A. Lewandowski

Department of Gastrointestinal and General Surgery, Wrocław Medical University, Wrocław, Poland

Interleukin-12 (IL-12) and interleukin-18 (IL-18) play an important role as immunomodulatory factors in cancer pathogenesis. The aim of the study was analyze changes of serum IL-12 and IL-18 concentrations in oesophageal squamous cell carcinoma patients depending on the progression of cancer. Materials and Methods: Blood samples were taken from 41 patients with oesophageal cancer: 5 women and 36 men, mean age 59 ± 9 years. 23 patients had surgical resection of oesophagus with II and III tumor stage, 18 patients with IV stage of cancer progression were treated by palliative procedures. The control group included 15 healthy blood donors: 4 female and 11 males, mean age 41 ± 6 years. The concentrations of IL-12 and IL-18 were determined by ELISA tests. Results: Serum IL-12 and IL-18 amounts detected in blood of oesophageal cancer patients were significantly higher in comparison to control group (p < 0.001). Serum IL-12 level was higher in patients with IV stage of the disease than in patients with II and III stages. Also serum IL-18 level was significantly higher in patients with IV stage in comparison to patients surgically treated (p < 0.05). Statistically significant differences were found in concentrations of IL-18 according to clinicopathological parameters such as: stage of cancer progression, tumor depth, lymph node metastasis (p < 0.05). Conclusions: Serum IL-12 and IL-18 levels are significantly higher in oesophageal cancer patients than in the healthy subjects. A relation between IL-18 content and cancer progression has been registered.

Key Words: interleukin-12, interleukin-18, oesophageal squamous cell carcinoma.

Oesophageal squamous cell carcinoma (ESCC) remains a devastating disease because it is usually not detected until it has progressed to an advanced stage. Modern imaging techniques are powerful tools in the detection, diagnosis, and staging of this disease. Early detection remains the elusive but essential goal of research. Only surgical resection at a very early stage has been shown to improve survival rates in patients with this disease [1].

The prognosis of patients with oesophageal tumors is poor, and elucidation of the molecular mechanisms of tumor growth and the establishment of informative biomarkers are thus under intensive investigation.

Interleukin-12 (IL-12) is a pleiotropic cytokine produced mainly by monocytes and natural killer (NK) cells, dendritic cells, Langerhans cells, keratinocytes and Kupffer cells. This interleukin stimulates Th1 lymphocyte development, proliferation and cytokine production. It has been shown to activate cytotoxicity in inflammatory diseases and against development many kinds of malignant tumours. The effect of IL-12 on tumors is mediated by NK cells, helper cells, cytotoxic T cells and is associated with interferon-γ (IFN-γ) production [2–4].

Interleukin-18 (IL-18), called interferon-γ-inducing factor, is a recently discovered cytokine that induces IFN-γ production in T cells and NK cells. It is synthesized by activated macrophages, Kupffer cells, dendritic cells, Langerhans cells and intestinal epithelial cells. IL-18 induces proliferation of activated T cells, activation of NK cells, secretion several cytokines and chemokines and participate in innate and acquired immunity [3, 5, 6]. An IL-18 receptor is present in natural killer cells and is induced by IL-12. Thus, IL-18 plays an important role in cooperation with IL-12, especially in Th1 response in antitumor immunity. At the tumor site, locally decreased IFN-γ production after decreased or abolished IL-18 production was correlated with an unfavourable outcome for patients with colon carcinoma [6, 7].

The role of IL-12 and IL-18 in activation of immune responses was observed in oesophageal [8], gastric [9, 10] and colon [6, 7] cancers. Numerous studies have demonstrated IL-12 and IL-18 ability to suppress tumor angiogenesis and growth [11–13].

The current study is the first to testing serum IL-12 and IL-18 levels in patients with oesophageal squamous cell carcinoma (ESCC). The aim of this study was to determine, whether serum IL-12 and IL-18 levels can be used as diagnostic markers in ESCC patients.

MATERIALS AND METHODS

Forty one patients with oesophageal squamous cell carcinoma were investigated between 2004 and 2005 in the Department of Gastrointestinal and General Surgery, Wrocław Medical University, Poland. The group comprised 5 women and 36 men aged 44–86 years (mean age 59 ± 9 years). Peripheral blood was obtained preoperatively from 23 consecutive patients who underwent surgical resection of oesophagus and from 18 patients who were treated by palliative procedures. The clinical stage was assigned according to the sixth edition of the TNM classification of the International Union Against Cancer (UICC) [14]. Cancer group included: 17 patients with stage II, 6 patients with stage III and 18 patients with stage IV; 17 cases of T2, 19 cases of T3 and 5 cases of T4; 9 cases of N0, 17 cases of N1 and 15 cases of N2 + N3; 27 cases of M0, 9 cases of M1 and 5 cases of M2. Control group consisted of 15 healthy blood donors: 4 female and 11 male, mean age 41 ± 6 years.
Blood samples were taken into sterile vacuum tubes and centrifuged at 3000 rpm for 10 min. Serum was stored at –20°C until use. IL-12 and IL-18 levels were determined by enzyme-linked immunosorbent assay (ELISA) (Bender MedSystem, Vienna, Austria). The ELISA kits measured protein levels using antibody p40 and p70 specific for IL-12, and anti-human IL-18 monoclonal antibodies. The samples were prepared and tested in duplicate according to the instructions.

The minimum detectable dose of IL-12 was < 15 pg/ml, and the upper limit of linearity was 2000 pg/ml. The minimum detectable dose of IL-18 was < 12.5 pg/ml, and the upper limit of linearity was 5000 pg/ml.

Data were expressed as mean ± SD, median and range. Analysis of distribution of data was carried on with Shapiro — Wilk normality test. Continuous variables were compared using Mann — Whitney U test and correlation coefficient was measured using R-Spearman test. Statistical significance was considered for p < 0.05 and p < 0.001.

Studies were approved by Ethics Committee University of Medicine in Wrocław, Poland.

RESULTS

Medians of serum IL-12 and IL-18 concentrations were significantly higher in ESCC patients than in the healthy subjects (p < 0.001) (Table 1). Serum IL-12 and IL-18 levels were significantly higher in surgically treated patients and in palliative treated patients in comparison with control group (p < 0.001) (see Table 1). Differences between values of IL-18 concentrations in operated and non-operated patients were statistically significant (p < 0.05).

| Table 1. Concentration of serum IL-12 and IL-18 in patients with squamous cell carcinoma of oesophagus in comparison with healthy individuals
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>IL-12 (pg/ml)</td>
<td>IL-18 (pg/ml)</td>
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<tr>
<td>Control group (n = 16)</td>
<td>Mean ± SD</td>
<td>Median (range)</td>
</tr>
<tr>
<td>ESCC patients (n = 41)</td>
<td>194.5 ± 166.7</td>
<td>195.0 ± 205.0</td>
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<tr>
<td>oesophagectomy (n = 23)</td>
<td>171.6 ± 166.7</td>
<td>155.2 ± 146.0</td>
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<tr>
<td>palliative procedure (n = 18)</td>
<td>218.6 ± 177.2</td>
<td>237.1 ± 239.95 ± 360.0</td>
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*p < 0.001 vs control group; †p < 0.05 vs oesophagectomy group.

Correlation of serum IL-12 and IL-18 levels with tumor features are presented in Table 2. Significant differences were not observed between serum IL-12 and serum IL-18 levels and patient’s age and sex. Medians of IL-18 levels were higher simultaneously with the increase in the degree of the development of cancer and were statistically significant between II and IV stage. As the depth of tumor invasion progressed, serum IL-12 and IL-18 amounts tended to increase and there were significant differences of IL-18 levels between T2 and T3 or T2 and T4 groups. Serum IL-18 level was significantly elevated (N0 vs N2+N3) when metastatic regional lymph nodes were present.

Serum IL-12 and IL-18 levels increased in the presence of distant metastasis, in case of IL-12 it was near to statistical significance.

| Table 2. Serum IL-12 and IL-18 levels with respect to clinical and pathological characteristics of oesophageal squamous cell carcinoma
<table>
<thead>
<tr>
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<tr>
<td>Age:</td>
<td>Median IL-12 (pg/ml)</td>
<td>Median IL-18 (pg/ml)</td>
</tr>
<tr>
<td>&lt; 60 years</td>
<td>163.55 p = 0.393</td>
<td>204.75 p = 0.490</td>
</tr>
<tr>
<td>&gt; 60 years</td>
<td>176.20</td>
<td>205.0</td>
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<tr>
<td>Sex:</td>
<td></td>
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<tr>
<td>Female</td>
<td>165.00 p = 0.293</td>
<td>99.00 p = 0.089</td>
</tr>
<tr>
<td>Male</td>
<td>171.45</td>
<td>212.00</td>
</tr>
<tr>
<td>Stage:</td>
<td>IL vs III: p = 0.244</td>
<td>III vs IV: p = 0.012</td>
</tr>
<tr>
<td>II vs IV: p = 0.060</td>
<td>131.00 vs III: p = 0.186</td>
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<tr>
<td>T2 vs T3: p = 0.094</td>
<td>150.00 vs N3: p = 0.003*</td>
<td></td>
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<tr>
<td>N2 vs N3:</td>
<td>144.75 vs N0: 131.00 vs N1: p = 0.061</td>
<td></td>
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<tr>
<td>N2 + N3:</td>
<td>207.75</td>
<td>227.30</td>
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<tr>
<td>N3:</td>
<td></td>
<td>N1 vs N2 + N3: p = 0.057</td>
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Distant metastasis (M): | M0 | M1 | M2 |
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<tr>
<td>157.50 M0 vs M1:</td>
<td>157.50 M0 vs M2:</td>
<td>157.50 M1 vs M2:</td>
</tr>
<tr>
<td>0.806</td>
<td>0.053</td>
<td>0.046</td>
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*p Statistical significance for p < 0.05; statistical analysis by Mann — Whitney U test.

The correlation coefficient for serum IL-12 and IL-18 levels was not statistically significant.

DISCUSSION

IL-12 and IL-18 have been found to act synergistically in their antitumor activity [3, 15, 16]. In the current study we tested serum IL-12 and IL-18 concentrations in blood of the patients with ESCC, where serum IL-12 and IL-18 levels were significantly higher in comparison with healthy subjects. We showed that ESCC patients surgically treated (stage II and III) had lower amounts of IL-12 and IL-18 than those in palliative treated patients (stage IV). Our investigations are in agreement with study of Tsuboi et al. [8], who demonstrated that serum IL-12 and IL-18 levels in patients with oesophageal carcinoma were significantly higher than in control group. These results suggest that IL-12 and IL-18 mediate the innate and adaptive antitumor immune response. It is believed that effects of IL-12 and IL-18 on tumors are mediated by natural killer cells, helper cells and cytotoxic T cells in association with IFN-γ production [3, 7, 8, 16]. Some authors reported that cancer immunotherapy with IL-12 results in eradication of disseminated disease in patients with colon cancer, malignant mesothelioma or lung cancer [17–21].

It has been demonstrated that production of IL-12 and IL-18 in oesophageal cancer correlated with survival rate of patients. The prognosis of patients who had a high level of both IL-12 and IL-18 was poor. These patients tended to show progressive tumor invasion [8–10].

We tested the relationship between concentrations of serum IL-12 or IL-18 and clinical and histopathological parameters in the patients with ESCC. We observed no significant differences in levels of IL-12 between the stages of ESCC. However, as the stage of cancer
progressed, serum IL-18 levels tended to increase and became statistically significant between stage II and IV. Kawabata et al. [9] demonstrated that in gastric carcinoma levels of IL-18 have been shown to be higher in stage II and III compared with stage I and IV of the disease. These results are in opposite to our study and other investigators [10], and did not apply to patients with other malignant tumors [7, 16]. Takahashi et al. [22] found that in advanced gastric cancer amounts of IL-12 and IL-18 increased and activated the production of hydrogen peroxide and the intracellular synthesis of IL-10 and IL-2 in comparison with early stage of the disease. In oesophageal carcinoma, serum IL-12 and IL-18 levels increased as the clinical stage progressed, although there were no significant differences between them [8]. Based on our study, we suggest that IL-18 production can be induced especially in response to the factors related to tumor development.

In the presence of IL-12 and IL-18 augment Th1 immune responses, including the production of IFN-γ [3, 23, 24]. Kawabata et al. [9] observed the reduction of IFN-γ production in gastric carcinoma patients and suggested that it can result in elevation of IL-18 levels by compensatory feedback mechanism.

Several investigators reported that IL-18 plays an important role in Th1 response and possibility in Th2 response. Another cytokines, such as IL-10, may affect the production and function of IL-18. This is a specific cytokine, in contrast to IL-12, which enhanced only Th1 responses [24].

Some studies report that tumor cells can contribute to an immunosuppressed state in the host. This strategy helps the tumor cells to escape from host — immune surveillance [25, 26]. It may account for the fact that in our study serum IL-12 levels were similar in all stages of cancer.

We demonstrated that as the depth of tumor invasion progressed, serum IL-12 and IL-18 tended to increase. We observed significant differences between serum IL-18 levels in T2 vs T3 and T2 vs T4 depth of tumor invasion. Tsuboi et al. [8] reported that values of IL-12 and IL-18 were the highest in T1 cases. In our study there were no ESCC patients with the T1 depth of tumor, but we observed the highest values for IL-12 in T4 cases and for IL-18 levels — in T3 and T4 cases. We have shown statistically significant differences in levels of IL-18 between N0 and N2 + N3 in regional lymph node metastasis.

Nakajima et al. [27] reported that lymphocyte infiltration around cancerous lesions is an important immune response. When the tumor remains in the mucosal or submucosal layer, there is a strong tendency for lymphocyte infiltration. Lymphocyte infiltration decreased as the depth of invasion progressed. In our study the values of IL-12 and especially IL-18 were high in N2 + N3 cases as a result of the immune response per clinical stage of cancer.

Some reports suggested that IL-18 inhibits angiogenesis, suppresses tumor growth and enhances antitumor responses. High level of IL-18 in advanced cancer can inhibit proangiogenic factors: VEGF-A, VEGF-C and others [11, 13, 28, 29]. It was recently reported that tumors could counterattack immune system by secreting IL-18. The serum IL-18 concentration was higher in metastatic cancer patients than in patients at initial stages of cancer [30]. Kim et al. [31] showed that recombinant human VEGF in gastric cancer cells can enhance IL-18 production and high level of IL-18 activated migration of cancer cells. This suggests that the IL-18 derived from cancer cells is involved in the metastatic process.

In conclusion, concentrations of serum IL-12 and IL-18 are significantly higher in ESCC patients than in the healthy subjects. Especially in case of IL-18 we have observed relation between interleukin level and cancer progression. We hope that IL-12 and IL-18 content could be used as diagnostic marker in detection of ESCC development.

REFERENCES


УРОВНИ ИНТЕРЛЕЙКИНА-12 И ИНТЕРЛЕЙКИНА-18 В СЫВОРОТКЕ КРОВИ БОЛЬНЫХ ПЛОСКОКЛЕТОЧНОЙ КАРЦИНОМОЙ ПИЩЕВОДА

Интерлейкин-12 (ИЛ-12) и интерлейкин-18 (ИЛ-18) играют важную роль в качестве иммуномодулирующих факторов в патогенезе локальных новообразований. Цель: анализ изменений уровней ИЛ-12 и ИЛ-18 в сыворотке крови больных плоскоклеточным раком пищевода при прогрессировании заболевания. Материалы и методы: образцы крови брали у 41 больного раком пищевода (5 женщин и 36 мужчин, средний возраст 59 ± 9 лет). У 23 больных в стадии II и III провели хирургическое удаление опухоли, 18 больным с IV стадией заболевания проводили паллиативное лечение. Контрольная группа состояла из 15 здоровых доноров (4 женщины и 11 мужчин, средний возраст 41 ± 6 лет). Концентрации ИЛ-12 и ИЛ-18 определяли иммуноферментным методом. Результаты: уровни ИЛ-12 и ИЛ-18 в сыворотке крови больных раком пищевода значительно выше, чем у здоровых людей (p < 0,001). Уровень ИЛ-12 выше у больных с IV стадией заболевания, чем у больных со стадиями II и III. Уровень ИЛ-18 в сыворотке крови больных с IV стадией заболевания выше, чем у больных, перенесших хирургическое вмешательство (p < 0,05). Статистически значимые различия в уровне ИЛ-18 зависели от различных клинико-патологических параметров (стадии заболевания, опухолевой инвазии, наличия метастазов в лимфатических узлах) (p < 0,05). Выводы: уровни ИЛ-12 и ИЛ-18 в сыворотке крови у больных раком пищевода значительно выше, чем у здоровых людей-доноров. Выявлена зависимость между содержанием ИЛ-18 и прогрессированием заболевания.

Ключевые слова: интерлейкин-12, интерлейкин-18, плоскоклеточный рак пищевода.