T-lymphocyte subsets and lipid peroxidation in relation to survival among advanced gastric cancer patients

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Background. Surgery is the most frequent primary treatment for gastric cancer. This treatment deepens the already existing injury of the immune system, and these infringements correlate with the degree of tissue trauma. It is generally accepted that reactive oxygen species could be important causative agents of cancer and are associated with the different steps of carcinogenesis either through immunological mechanisms. Little is known about the changes that occur in the antioxidant and lipid peroxidation levels in response to surgical trauma.

The aim of this study was to evaluate changes of the parameters of immune and oxidant-antioxidant status during surgical treatment of gastric cancer patients and to evaluate the effect of these parameters on survival after gastric cancer surgery.

Materials and methods. Twenty two patients with histologically confirmed gastric carcinoma in stage III–IV were eligible for inclusion in the present study (14 patients in stage III and 8 in stage IV). The median age of patients was 62 years (range 44–75 years). There were 14 males and 8 females.

The indices of cellular immunity were measured by immunofluorescence methods. Indirectly the state of the antioxidant system is reflected by the intensity of lipid peroxidation, which was evaluated by the concentration of peroxidation products – diene conjugates and malondialdehyde.

Results. Analysis of the data showed that the overall survival of patients with gastric cancer stage III–IV, pre-surgical CD3+ lymphocyte levels >0.8·10^9/l was significantly higher (P= 0.0201, log-rank test) compared to patients with pre-surgical CD3+ lymphocyte levels <0.8·10^9/l. The overall survival of patients with pre-surgical CD4+ lymphocyte levels >0.25·10^9/l was significantly higher (P = 0.014, log-rank test) compared to patients with pre-surgical CD4+ lymphocyte levels <0.25·10^9/l. The overall survival of patients with pre-surgical CD8+ lymphocyte levels >0.3·10^9/l was significantly higher (P = 0.018, log-rank test) compared to patients with pre-surgical CD8+ lymphocyte levels <0.3.

Analysis of the parameters of the lipid peroxidation process in relation to the survival indicates a significantly lower level of MDA concentration after surgery (9.2 and 13.2 nmol/ml respectively, p = 0.0257), while decrease of DK concentration after surgery was insignificant (6.2 and 7.3 nmol/ml respectively, p = 0.068).

Conclusions. This study suggests that higher levels of T lymphocyte subsets absolute number before surgery have a beneficial effect on the overall survival of advanced gastric cancer patients. Malondialdehyde concentration decreased after surgery of gastric cancer patients in stage III and IV. Lower levels of the parameters of lipid peroxidation after surgery may play a positive role in the survival of gastric cancer patients.

Key words: gastric cancer surgery, immune system, lipid peroxidation, survival
INTRODUCTION

According to the available cancer epidemiology data, the annual number of new cases increased significantly in the developing world. Gastric cancer is the leading cause of cancer-related mortality in the world as well as in Lithuania. Cancer arises frequently on the background of immunodeficiency and is associated with cellular immunodeficiency (1). Patients with gastric cancer have a variety of immunological abnormalities and exhibit a poorly functioning immune system (1, 2), manifested by a decreased T-cell proliferation, reduced CD4/CD8 ratio (3) and a deficient production of T-helper cytokines (2, 4). Malignant tumors have been suggested to cause an oxidative disorder in the antioxidative defense system (5–7). The level of lipid peroxidation products, especially malondialdehyde (MDA) level in blood, are considered to be the criterion for the oxidative status of the entire human organism (8).

Surgery is the most frequent primary treatment for gastric cancer. This treatment deepens the already existing injury in the immune and antioxidative systems and infringements correlated with the degree of tissue trauma (9, 10). The markers reflecting the immunological status and the level of oxidative stress of patients after surgical treatment might be of value in the prognosis of postoperative complications as well as of survival of patients.

The aim of this study was to evaluate the values of the parameters of protective systems of the gastric cancer patients and to evaluate the effect of the immune and oxidant–antioxidant status on the survival of patients after gastric cancer surgery.

MATERIALS AND METHODS

Twenty-two patients (14 males and 8 females) with histologically confirmed gastric carcinoma in stage III–IV were eligible for inclusion in the present study (14 patients in stage III and 8 in stage IV).

17 patients had been treated by resection and 5 by gastrectomy. The median age of patients was 62 years (range 44–75 years).

The patients included in this study were selected according to their odd number of surgical history. They had preoperative WBC count >3.0 · 1012/l, hemoglobin >100 g/l, leucocytes >3.0 · 10⁹/l, platelets >180 · 10⁹/l. Venous blood of cancer patients was tested before surgery (1st analysis) and 14 days after surgery (2nd analysis).

The indices of cellular immunity determined for all the investigated persons were the following: leucocyte number, total lymphocyte, monocyte and neutrophile percentage and absolute number. By immunofluorescence methods the percentage and absolute number of total T lymphocyte population (CD3⁺), T helpers (CD4⁺), T cytotoxic cells (CD8⁺), immunoregulation index (CD4⁺ / CD8⁺), B lymphocyte (CD20⁺), NK cells (CD16⁺) (Sorbent, Russia) were measured.

Indirectly the state of the antioxidant system is reflected by the intensity of lipid peroxidation, which was evaluated by the concentration of the peroxidation products, diene conjugates (DK) and malondialdehyde (MDA). Determination of DK is based on its extraction in heptane-isopropanol mixture and of MDA on the reaction with thiobarbituric acid.

The statistical analysis of cellular immunity parameters was performed and the intensity of lipid peroxidation determined by two-tailed, paired Student’s t test.

The survival was calculated from the date of operation to the date of death or the last date the patient was known to be alive. All patients were followed-up via the Lithuanian Cancer Registry. The influence of the parameters of lymphocyte subsets and lipid peroxidation on survival was evaluated by distributing the patients into subgroups according to the gradual cut-off levels in each lymphocyte subset and lipid peroxidation parameters. The survival of patients was analyzed using the Kaplan–Meier method. The difference between survival curves was determined using the log-rank test. For a more precise survival analysis the Cox regression method was also used (in order to reject the influence of patient’s age, sex, gastric cancer disease stage). The significance was accepted at P < 0.05.

RESULTS

The cellular immunity reflecting parameters (total leucocyte, monocyte, total lymphocyte, and CD3⁺, CD4⁺, CD8⁺, immunoregulation index CD4⁺ / CD8⁺, CD20⁺, CD16⁺) were measured.

Graph 1. Dependence of overall survival of gastric cancer patients on the number of CD3⁺ lymphocytes before surgery

Group 1 - CD3⁺ lymphocytes ≤ 0.8 · 10⁹/l, group 2 - CD3⁺ lymphocytes > 0.8 · 10⁹/l.
percentage and absolute number) 14 days after gastric cancer surgery (2nd analysis) were compared to the related parameters in research before treatment (1st analysis). The parameters of cellular immunity were not changed significantly after gastric cancer surgery.

The Cox analysis has shown that the higher absolute number of lymphocytes may be associated with a longer survival of gastric cancer patients in stage III and IV (P = 0.023). The survival of cancer patients was also analysed by gradually testing different cut-off numbers of total lymphocytes and lymphocyte subsets. The analysis showed that the overall survival of patients with III–IV gastric cancer stage and pre-surgical $CD_3^+$ lymphocyte levels > 0.8·10^9/l was significantly higher (P = 0.0201, log-rank test) compared to patients with the pre-surgical $CD_3^+$ lymphocyte levels < 0.8·10^9/l (Graph 1), and their 3-year survival was 71% and 7% respectively. The Cox analysis has confirmed this tendency for $CD_3^+$ lymphocyte (P = 0.006). The overall survival of patients with pre-surgical $CD_3^+$ lymphocyte levels > 0.25·10^9/l was significantly higher (P = 0.014, log-rank test) compared to patients with pre-surgical $CD_3^+$ lymphocyte levels < 0.25·10^9/l (Graph 2), so the 3-year survival of patients with stage III–IV gastric cancer was 46% and 0% respectively. The overall survival of patients with pre-surgical $CD_4^+$ lymphocyte levels > 0.3·10^9/l was significantly higher (P = 0.018, log-rank test) compared to patients with pre-surgical $CD_4^+$ lymphocyte levels < 0.3·10^9/l (Graph 3), so the 3-year survival was 54.5% and 0% respectively. The Cox analysis confirmed these tendencies: $CD_4^+$ lymphocytes (P = 0.011), and $CD_8^+$ lymphocyte (P = 0.0075).

The parameters of lipid peroxidation products (malondialdehyde and diene conjugates) were studied in gastric cancer patients before and after operation. A significantly lower level of MDA concentration was observed after surgery (9.2 nmol/ml), while before surgery it was higher (13.2 nmol/ml) (P = 0.0257). The decrease of DK concentration after surgery was insignificant – 6.2 and 7.3 nmol/ml respectively (P = 0.068). A nalysis of the parameters of the lipid peroxidation process in relation to survival indicates that after surgery the value of the MDA parameter, which has a positive influence on the survival of gastric cancer patients, was 8 ≤ nmol/ml (P = 0.0107; Graph 4). According to the Cox analysis, irrespective of disease stage, patient’s sex and age, there was a tendency

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**Graph 2.** Dependence of overall survival of gastric cancer patients on the number of $CD_3^+$ lymphocytes before surgery

**Graph 3.** Dependence of overall survival of gastric cancer patients on the number of $CD_8^+$ lymphocytes before surgery

**Graph 4.** Dependence of survival of gastric cancer patients on MDA concentration after surgery

**Graph 5.** Dependence of survival of gastric cancer patients on DK concentration after surgery
that the survival depended on MDA values of the 1st and 2nd analysis (P = 0.061 and 0.098 respectively). DK concentration had almost no influence on the survival of gastric cancer patients after surgery (Graph 5), although the 2nd analysis of DK had a tendency (P = 0.068) to exert a positive effect on the survival.

**DISCUSSION**

Thus, analysis of the obtained data has shown that the parameters of cellular immunity (total leukocyte, monocyte, total lymphocyte, and CD4+, CD8+, CD16+; immunoregulation index (CD4+/CD8+), CD20+, CD14+; percentage and absolute number) were only insignificantly changed after gastric cancer surgery. This fact demonstrates the absence of the influence of surgical treatment on cellular immunity parameters of gastric cancer patients, or these parameters returned to the initial level within 14 days.

Although we do not completely understand the mechanisms that underlie the specific immunologic alterations, it is clear that both functional and quantitative defects of immunity develop with cancer, especially in advanced stages.

Analyzing the dependence of survival on immunological indices of gastric cancer patients, a significant dependence of survival was determined on the absolute pre-treatment number of lymphocyte subpopulation (CD4+ lymphocyte levels > 0.8·109/l, CD8+ > 0.25·109/l, CD19+ > 0.3·109/l). The Cox analysis has shown that the highest absolute number of lymphocyte subpopulation may be associated with a longer survival of gastric cancer patients in stage III and IV. Other authors underline that immunosuppression is associated with CD4+, CD8+, CD16+; T-cell level depression, and it worsens the prognosis for patients with stage III gastric cancer. The 5-year disease-free survival rates of patients with stage III gastric cancer were poor at lower values of CD4+ and CD8+ T-cells (11, 12).

Data on the lipid peroxidation process in gastric cancer patients show that the significantly decreased level of the lipid peroxidation product - malondialdehyde after operation has a positive influence on the survival of gastric cancer patients in stage III-IV. The Cox analysis shows that the survival depends on MDA values before and after operation, but it is necessary to note that it depends more on the MDA concentration before operation. The level of lipid peroxidation products, diene conjugates, had almost no influence on the survival; only 2nd analysis had a tendency to exert effect on the longer survival of gastric cancer patients in stages III-IV.

**CONCLUSIONS**

1. The parameters of cellular immunity before surgery and 14 days after surgery statistically did not differ.
2. This study suggests that higher levels of the absolute number of T lymphocyte subsets before surgery have a beneficial effect on the overall survival of gastric cancer patients at advanced stages of the disease.

3. Malondialdehyde concentration decreased after surgery in gastric cancer patients in stage III and IV. Lower levels of the parameters of lipid peroxidation after surgery may play a positive role in the survival of gastric cancer patients.

**REFERENCES**

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Medžiaga ir metodai. Ištyrime 14 ligonius, sergančius III skrandžio vėžio stadija, ir 8 ligonius – IV skrandžio vėžio stadija.

Nustatyti įvairios imuniteto rodikliai: leukocito, neutrofilo, monocito, bendrøjo limfocito, limfocito subpopuliacija (CD3, CD4, CD8, CD20, CD16) procentinis ir abso- liutus skaičius, taip pat lipidø peroksidacijos produktø – dijieniniø konjugatø (DK) ir malono dialdehido (MDA) – koncentracija ligoniø periferiniame kraujyje prieð operacijo ir praëjus 14 dienø po operacijo.

Rezultatai. Analizës duomenimis, mûsø tirtieji įvairios imuniteto rodikliai, praëjus 14 dienø po operacijo, statistiðkai nepakito. Galima manyti, kad operacija neturëjo áta- kos įvieno imuniteto rodikliams arba jie atsistiðtê. Taëau ilgai gyveno tie ligoniai, kuriems prieð operacijo buvo nustatytas CD3 limfocitø absoliutus kiekis >0,8·10⁹/l (P = 0,0201, log-rank testu), CD4 - >0,25·10⁹/l (P = 0,018, log-rank testu), CD8 - > 0,25·10⁹/l (P = 0,0201, log-rank testu). Kiti įvieno imuniteto rodikliai ligoniø gyvenimo trukmë atakos neturëjo.

Sumaþëjusi lipidø peroksidacijos produkto MDA koncentracija (P = 0,0107), praëjus 14 dienø po operacijo, prailgino skrandžio vėžiu serganæio ligoniø gyvenimo trukmë.

Išvados. Ligoniai, sergantys vëlyviomis skrandžio vë- þio stadijomis, kuriems prieð operacijà buvo nustatytas CD3 limfocitø absoliutus kiekis > 0,8·10⁹/l, CD4 - >0,25·10⁹/l, CD8 - > 0,25·10⁹/l, gyvena ilgaiu. Lipidø peroksidacijos produkto MDA koncentracijos sumaþëjimas po operacijos prailginio skrandžio vëþiu ser- ganæio ligoniø gyvenimo trukmë.

Raktąpadëjai: skrandþio vëþys, chirurginis gydymas, imuninë sistema, lipidø peroksidacija, gyvenimo trukmë.