

Combined treatment for gastric cancer: Immunological approach

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ABSTRACT

Background/Aims: Gastric cancer is one of the most common oncological diseases. It can develop in any part of the stomach and spread to other organs, especially the esophagus, lungs, and liver. The aim of our study was to investigate the effectiveness of our proposed therapy.

Materials and Methods: Our research promises more effective neoadjuvant therapy, including immunotherapy and multi-agent chemotherapy. Of the 62 patients involved in our study, 32 underwent neoadjuvant chemotherapy in combination with surgery, whereas the rest underwent neoadjuvant chemoimmunotherapy with surgery.

Results: Investigation of T-cell-mediated and humoral immunity in patients with gastric cancer over the course of treatment found a reduction of the main indices of cell-mediated and humoral immunity in the patients who underwent standard therapy, which greatly caused a decline of antitumor, anti-infective, and antitoxic protection of the patients' organisms.

Conclusion: This study can contribute to the development of new therapies for gastric cancer as well as other types of cancer.

Keywords: Oncology, *Helicobacter pylori*, malignant neoplasm, mortality structure, immunotherapy, combined neoadjuvant therapy

INTRODUCTION

According to the World Health Organization (WHO), oncological diseases are one of the main causes of morbidity and mortality in the world. In 2013, the WHO issued a Global Action Plan for the Prevention and Control of Non-communicable Diseases 2013-2020, which was aimed at a 25% relative reduction in premature mortality from cancer. Certain voluntary targets are especially important for cancer prevention; for instance, a 30% reduction of tobacco use in 2014-2025. It is estimated that cancer incidence will increase by about 70% in the next 20 years (1).

About one-third of deaths from cancer are caused by five main behavior- and diet-related risk factors, such as high body mass index, insufficient intake of fruits and vegetables, lack of physical activity, use of tobacco, and consumption of alcohol. Infections that cause cancer, such as HBV/HCV, *Helicobacter pylori*, and HPV, are responsible for 20% of deaths from cancer in countries with low and average wage rates (1).

Most new cases of cancer are registered in African and Asian regions as well as in Central and South America. The WHO predicts an increase in the incidence of oncological diseases (2-4).

In 2012, Kazakhstan adopted a program for cancer care, which established the Specialized Research Center and started the development of the necessary healthcare system. Mortality from oncological diseases holds the second place in the mortality structure in the Republic of Kazakhstan. Annually, approximately 17,000 people die of cancer, 42% of whom are working-age people (5-7).

Information about the molecular pathogenesis of gastric cancer allows conducting directed search for new approaches to the medicinal treatment of gastric cancer. Expression of apoptosis indicators with gastric cancer is related to the morphological features of the tumor, which may have prognostic value when predicting the course of the disease. However, data obtained by different authors are ambiguous (8).

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At present, gastric cancer is only fourth among oncological pathologies in terms of prevalence after breast cancer, skin cancer, and lung cancer (9,10). In 2014, 34,352 new cases of malignant neoplasms were registered in the Republic of Kazakhstan (33,029 in 2013), 45.5% (45.5% in 2013) of which were in men, whereas 55.0% (54.5% in 2013) were in women.

The most widespread forms of cancer are lung and stomach cancer among men and breast and skin cancer among women (9).

Men are affected more often than women are; standardized global incidence indices are about 22 cases and 10 cases per 100,000 persons per annum, respectively; however, this difference is noted only for the intestinal form of gastric cancer (11). The incidence of gastric distal cancer has a tendency to decrease, whereas that of gastric cardiac cancer remains unchanged and even slightly increases (12). Gastric cancer is a particularly common pathology in East Asia (Japan and Korea), South America, and Eastern Europe, but it is less pronounced (11,13).

Nowadays, most experiments and clinical studies decisively indicate an unbalanced immune system with oncological pathology (14). Both Kazakh and foreign researchers established the existence of a reciprocal link between the immune system and the tumor, which determines the course and outcome of the neoplastic process and the state of the immune response in the patient's organism (15).

Currently, preclinical and clinical trials of drugs intended for gastric cancer treatment are being conducted. Diagnostics and treatment of gastric cancer in the Republic of Kazakhstan have improved owing to the implementation of screening programs according to the Decree of the Minister of Healthcare of the Republic of Kazakhstan dated March 16, 2011, No. 145 and the Decree of the Minister of Healthcare of the Republic of Kazakhstan dated January 8, 2013, No. 8 "On the Implementation of Screening for Early Detection of Esophageal Cancer, Gastric Cancer, Liver Cancer, and Prostate Cancer in Pilot Regions" (16). Five institutional reforms and about a 100 concrete steps for their realization were outlined in the National Plan, which enhanced the stability of the healthcare system on the basis of solidary responsibility of the government, employers, and citizens. Primary aid has become the cornerstone of national healthcare in regard to

the prevention and early treatment of diseases. The feature of the accessibility and quality of medical services in the Republic of Kazakhstan lies in the principles of corporate management and the implementation of progressive standards of medical services (5).

Gastric cancer is an example of obvious progress in medical oncology. Epidemiological studies determined the main risk factors and enabled formulating a series of recommendations regarding its treatment.

MATERIALS AND METHODS

The effectiveness of combined neoadjuvant therapy-multi-agent chemotherapy + immunotherapy was studied on patients with locally advanced gastric cancer. Of the 62 patients who underwent treatment, 32 underwent "neoadjuvant multi-agent chemotherapy + surgery" treatment, whereas the remaining 30 underwent "neoadjuvant chemoimmunotherapy + surgery" (17,18). Multi-agent chemotherapy was conducted as follows: 75 mg/m² docetaxel (EBEWE Pharma GmbH, Austria) on day 1 + 500 mg/m² 5-phthoruracil (Nantong Jinghua Pharmaceutical Co., China) on days 2-5 + 50 mg/m² calcium folinate (Hospira, USA) on days 2-5. The immunotherapy subgroup took 20 mg of thymalin (Samson JSC, Russia) for 6-10 days after the multi-agent chemotherapy course. The control groups included patients who underwent surgery only and people without the studied diseases.

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of The Third Affiliated Hospital of Higher School of Public Health. All the patients gave their informed consent prior to enrollment.

Statistical analysis

Statistical processing was carried out using the program STATISTICA 6.0 (StatSoft, Palo Alto, California, USA).

RESULTS

The immune status of patients in the studied groups was examined; the results are presented in Table 1.

Table 1 shows that in healthy persons, the concentration of interleukin-2 (IL-2) in the blood serum was 213.5±0.1 pg/mL, whereas in patients with gastric cancer before the treatment, the IL-2 level was significantly higher 289.3±7.9 pg/mL, which may be related to an enhanced operation of the immune system due to tumor growth.

Table 1. Univariate analysis for risk factors of mortality in cirrhotic patients with UGIB

Patient group	Therapy stage	IL-2	TNF α	IFN γ
Control (healthy) n=25	-	213.5 \pm 0.1	18.2 \pm 1.1	14.7 \pm 1.9
	in autologous blood preparation mean \pm s.d.	285.6 \pm 1.0*	34.5 \pm 1.2*	25.4 \pm 2.6*
Gastric cancer patients n=128	Before surgery mean \pm s.d. (n=66)	289.3 \pm 7.9**	23.3 \pm 0.2	25.3 \pm 3.7**
	in autologous blood preparation mean \pm s.d.	335.8 \pm 8.5***	32.5 \pm 1.2***	28.6 \pm 2.4***
	After chemotherapy + surgery mean \pm s.d. (n=32)	254.7 \pm 8.1****	25.4 \pm 1.3	18.8 \pm 3.6
	After chemoimmunotherapy + surgery mean \pm s.d (n=30)	299.8 \pm 7.1	26.6 \pm 9.3	29.4 \pm 3.4

*: index between healthy persons and those with self-stimulatory behavior

**: index between healthy persons and gastric cancer patients before treatment

***: index before treatment and in the autologous blood preparation

****: index before treatment and after chemotherapy + surgery

After in vitro contact with thymalin and holding in a thermostat, the lymphocytes of healthy persons increased the IL-2 level to 285.6 \pm 1.0 pg/mL; the index grew by 33.8%. In patients with gastric cancer, lymphocytes were stimulated to release cytokines, including IL-2, up to 335.8 \pm 8.5 pg/mL. After chemotherapy, the mean IL-2 level in patients with gastric cancer was 254.7 \pm 8.1 pg/mL, it dropped by 12%, whereas in the group of patients who underwent chemotherapy with simultaneous intramuscular administration of thymalin, the concentration of cytokine increased by 3.7%.

The lack of a reliable effective immunotherapy is probably related to the uncertainty surrounding the point at which it should be conducted (before and/or after surgery). Examination of autologous blood preparations found that thymalin was capable of stimulating the immunity via active release of studied cytokines.

The in vitro study of the level of tumor necrosis factor alpha (TNF α) in the blood serum of patients with gastric cancer and healthy persons yielded the following results. Patients with gastric cancer had a slightly increased level of TNF α (23.3 \pm 1.5 pg/mL) versus healthy persons from the control group (18.2 \pm 1.1 pg/mL), i.e., 13.8% higher, which did not reach statistically significant levels.

The degree of stimulation of lymphocytes and other immune system cells using thymalin in the autologous blood of healthy persons and patients with gastric cancer was compared on the basis of the quantitative indices of the TNF α level in pg/ml. The stimulation capacity was similar: the TNF α level was 34.5 \pm 1.4 pg/mL in healthy persons

and 32.5 \pm 1.3 pg/mL in patients with gastric cancer; the obtained results were equivalent.

The chemotherapy + surgery group displayed an insignificant reduction of the TNF α level, whereas with chemoimmunotherapy + surgery TNF α level increased.

The level of IFN γ in patients with gastric cancer before treatment was 1.7 times higher than that in healthy persons (14.7 \pm 1.9 pg/mL). The thermostat incubation of the autologous blood preparation of healthy persons with thymalin found a more than 1.7-time increase in the cytokine level (25.4 \pm 2.6 pg/mL). The concentration of cytokine in the autologous blood preparation of patients with gastric cancer after thymalin stimulation was 28.6 \pm 2.4 pg/mL, which is indicative of a pronounced stimulating effect of the immune drug.

The cell-mediated and humoral immunity was studied over the course of treatment in patients with gastric cancer (neoadjuvant chemotherapy and chemoimmunotherapy) and in healthy persons via blood cell differentiation into cells in the immune system. Table 2 shows the main indices of T-cell-mediated immunity in patients with gastric cancer over the course of treatment in percentage.

The findings of the comparison of T-cell-mediated immunity in healthy persons and patients with gastric cancer before the treatment were typical for patients with malignant neoplasms—T-cell deficiency with reduced T helper cell subpopulation, increased rate of immature lymphocytes, and disruption of the immune regulation

Table 2. Main indices of T-cell-mediated immunity in patients with gastric cancer over the course of treatment (in percentage)

Patient group	Therapy stage	IL-2	TNF α	IFN γ
I-control (n=32)	29.6 \pm 0.4*	24.5 \pm 1.6	1.21 \pm 0.02*	54.6 \pm 0.6*
chemotherapy + surgery	26.4 \pm 0.8	23.6 \pm 1.3	1.11 \pm 0.01	50.4 \pm 0.6**
II-experimental (n=30)	29.7 \pm 0.5*	23.3 \pm 1.1	1.27 \pm 0.02*	52.2 \pm 0.5*
chemoimmunotherapy + surgery	28.2 \pm 0.5	22.7 \pm 1.5	1.25 \pm 0.01	50.6 \pm 0.9
In health (n=25)	46.3 \pm 0.7	21.4 \pm 0.3	2.16 \pm 0.08	67.7 \pm 0.8

*: difference between the index before treatment and in health is significant, $p < 0.05$

** : difference between the index before and after treatment is significant, $p < 0.05$
 numerator-index before treatment, denominator-index after treatment

ratio (T helper cells/regulatory T cells). Patients with gastric cancer also had initial T helper cell deficiency, which caused a decline of the regulatory functions of the immune system, including the detoxifying and antitumor function. Over the course of treatment, immunity indices in the gastric cancer group who underwent neoadjuvant chemoimmunotherapy were safer.

Obtained data show that the initial level of T helper cells was reduced in all observed patients, which was an absolute indication for the conduction of immune correction therapy.

The analysis of immunograms in the postoperative period in the control group found an absolute and relative T-cell deficiency with a disruption of the immunoregulatory function ($p \leq 0.05$). The experimental group showed a tendency of reduction of the main indices of cell-mediated immunity.

Table 3 shows that the comparison of indices in patients with gastric cancer before the treatment and healthy persons found a significantly lower synthesis of IgM and G antibodies in patients with gastric cancer before treatment. The concentration of macromolecular complexes (MMC) before treatment exceeded the normal level by 1.7 times in both groups, which also characterizes the degree of endotoxin binding by antibodies. After treatment, the control group showed a tendency of reduced IgA and IgG antibody count. In the experimental group, the discovered tendency was that of increased synthesis of IgM, IgA, and IgG antibodies ($p < 0.05$) after treatment in patients with gastric cancer. IgG antibodies have a direct antitumor effect. The concentration of MMC in the experimental group dropped from 5.45 ± 0.07 to 4.69 ± 0.06 ($p < 0.05$) over the course of treatment.

DISCUSSION

Tumor-caused disorders in the immune system can be exacerbated by the effect of various antineoplastic measures. Literature describes both qualitative and quantitative alterations that depend on the scope and nature of surgery (19).

All currently known chemotherapeutic agents affect actively proliferating cells and damage not only tumor cells but also healthy tissues of the patient's organism, primarily hematopoietic organs, where immunocompetent cells grow, which causes additional immunosuppression in the already unbalanced immune system (20). Thus, operational trauma and combined therapy using cytostatic agents exacerbates the initial immunodeficiency in patients with cancer, thus reducing the effectiveness of treatment. This necessitates immune system correction measures with regard to the degree and focus of registered disorders (21).

The literature review shows that drugs of varying origin and composition, which have the common property of nonspecific enhancement of the organism's immune response, are used nowadays to conduct immunotherapy. In clinical practice, the most common immunomodulators are of polysaccharide and lipopolysaccharide nature: proper-mil, zymosan, prodigiosan, pyrogenal, and thymalin (22). So far, these drugs are best suited for immune system stimulation because their effect on the immune system is not related to the disruption of phagocytic activity of neutrophils and macrophages.

The presence of a tumor (gastric cancer) in the organism has a major suppressive effect on the immunity in and of itself, which creates problems for neoadjuvant immunotherapy. Tumors have developed strategies to successfully

Table 3. Humoral immunity indices in patients with gastric cancer over the course of treatment

Patient group	Therapy stage	IL-2	TNF α	IFN γ
I-control	2.45 \pm 0.08*	0.85 \pm 0.15*	9.46 \pm 0.6*	5.65 \pm 0.03*
chemotherapy + surgery	2.28 \pm 0.09	0.80 \pm 0.12	8.68 \pm 0.4	6.52 \pm 0.05**
II-experimental	2.38 \pm 0.09*	1.04 \pm 0.03*	10.51 \pm 0.15*	5.45 \pm 0.07*
chemoimmunotherapy + surgery	2.46 \pm 0.10	1.05 \pm 0.04	10.74 \pm 0.16	4.69 \pm 0.06**
In health	3.77 \pm 0.06	1.21 \pm 0.06	12.10 \pm 0.16	3.37 \pm 0.05

*: difference between the index before treatment and in health is significant, $p < 0.05$

** : difference between the index before and after treatment is significant, $p < 0.05$
 numerator-index before treatment, denominator-index after treatment

evade the host immune system; various molecular and cellular mechanisms responsible for tumor evasion have been identified. Some of these mechanisms target immune antitumor effector cells. Dysfunction and apoptosis of these cells in the tumor-bearing host creates an immune imbalance that cannot be corrected by immunotherapies aimed only at the activation of antitumor immune responses. Reversal of existing immune dysfunction(s) and normalization of lymphocyte homeostasis in patients with cancer needs to be a part of future cancer immunotherapy. An attempt was made to develop a new method of immunotherapy, which would be able to showcase its activity, affect the general state of the immunity, and enhance cytokine formation, which would have a direct cytolytic effect on the neoplastic cells and metastases (23).

In this research, we studied the effect of chemicals in combination with surgery or without it on the development of the disease. However, there is also an alternative to this method-gene therapy. Recent studies have shown that survivin controls the expression of this gene, which can reduce the viability of cancer cells and inhibit the tumor growth (22). This can delay tumor growth in patients with more advanced cancer and allow the doctors to remove it more effectively.

In this study, docetaxel and thymalin were used in neoadjuvant therapy, but there are also data from other authors of the study. They investigated the effects of other substances on the treatment of gastric cancer. In particular, trastuzumab emtansine, or T-DM1, had a stronger effect on the development of tumors when compared with lapatinib and capecitabine. It should be noted that such treatment can also use cytotoxic T-lymphocytes. Researchers also used survivin, which resulted in cells acquiring specificity for lysis against cancerous cells (24). In

addition, previous studies used cancer-specific immunotherapy based upon mitotic centromere-associated kinesin (MCAK), a new cancer antigen (25).

In healthy persons, immunocompetent cells under the effect of thymalin enhanced the release of TNF α when compared with patients with gastric cancer; the cytokine level in patients with gastric cancer was 28% higher than that in healthy persons; the combination of said forms of therapy with immunotherapy indicated a tendency of cytokine count growth. Investigation of T-cell-mediated and humoral immunity in patients with gastric cancer over the course of treatment found a reduction of the main indices of cell-mediated and humoral immunity in patients with gastric cancer who underwent standard therapy, which greatly caused a decline of antitumor, anti-infective, and antitoxic protection of the patients' organisms. Inclusion of immunotherapy in the combined treatment of gastric cancer facilitates the normalization of cell-mediated and humoral immunity in patients.

Data from this study can be used in further development and search for new approaches to adjuvant treatment of gastric cancer. After analyzing approaches to solving the problem of the effectiveness of our therapy and that of other researchers, it is possible to develop a new method of treatment of this disease. This will greatly reduce the severity of the disease in patients and will help effectively remove primary and secondary negative manifestations of cancer.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the Higher School of Public Health.

Informed Consent: Written informed consent was obtained from patients who participated of this study (Cooperation Agreement No: 14/1 from 28-12-2015).

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