

**NAO "Astana Medical University"**

**ABSTRACT**  
**of the dissertation work**  
**for the degree**  
**of Doctor of Philosophy PhD**

**Topic: Molecular genetic and microbiological aspects**  
**of stomach cancer in Kazakhstan**

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## ANNOTATION

**of the dissertation work of Shtephanov Ivan Ivanovich on the theme: "Molecular genetic and microbiological aspects of stomach cancer in Kazakhstan", submitted for the degree of Philosophy Doctor (PhD) in the specialty 6D110100 – Medicine**

### **The relevance of research**

Cancer is one of the leading causes of death in the world; thus, in 2019, 9.6 million people died from this disease.

Gastric cancer (GC) in the structure of oncological pathology ranks third in terms of mortality in the world. Approximately 1.03 million people develop stomach cancer each year, with a death rate of 783,000 (2018).

In Kazakhstan, the death rate from neoplasms in 2019 is 75.5 people per 100 thousand of the population, while the prevalence of all cases of cancer (without skin cancer) is 174.8 people per 100 thousand of the population. According to the statistics "Indicators of the Oncological Service of the Republic of Kazakhstan", the mortality from stomach cancer in the Republic of Kazakhstan for 2019 is 9.4 cases per 100 thousand population, the incidence is 14.4 cases per 100 thousand population.

Gastric cancer is a multifactorial disease, the etiological factors of which are: dietary habits, environmental factors, bad habits (alcohol, smoking), infectious and hereditary factors.

Research in the field of biological chemistry, molecular biology and genetics, practical medicine opens up new possibilities for the diagnosis, prognosis, treatment and prevention of multifactorial diseases based on the characteristics of the genetic predisposition to the disease. It has been proven that approximately 5-10% of cases of malignant neoplasms of the stomach have a hereditary predisposition.

According to the revised criteria for CDH1 testing (taking into account the first degree and second degree of relationship), updated in 2015 by the International Gastric Cancer Consortium (IGCLC - International Gastric Cancer Linkage Consortium), familial cases include: 1) families with two or more patients with gastric cancer at any age, one confirmed hereditary diffuse gastric cancer; 2) individuals with hereditary diffuse gastric cancer under the age of 40; and 3) families diagnosed with hereditary diffuse gastric cancer and lobular breast cancer (one diagnosis before the age of 50). In addition, CDH1 testing is required in patients with bilateral or familial lobular breast cancer under 50 years of age, patients with diffuse gastric cancer and cleft lip or palate, and individuals with precursors of signet ring cell carcinoma.

Emerging data from early studies indicate that single nucleotide polymorphisms (SNP) of the genes of tumor necrosis factor (TNFa) interleukin 10 (IL10), tumor protein p53 (TP53) [8] and differentiation cluster 14 (CD14) can determine individual predisposition to stomach cancer. The TNF and IL10 cytokine genes, CD14, a gene related to innate immunity, and the tumor suppressor gene TP53 are multifunctional genes involved in the development and progression of many malignant tumors

**The purpose of the study:** Determination of the role of genetic, molecular changes and microbiological factors in the risks of gastric cancer and related clinical course features in the studied population.

**Research objectives:**

1. Study of polymorphisms of TNF $\alpha$ , IL1B, IL10, TP53 and CD14 genes, germinal mutations of the CDH1 gene and identification of an association with the risk of stomach cancer in the population of the Republic of Kazakhstan.

2. To study the expression of the E-cadherin protein in patients with gastric cancer and to assess the relationship between the level of expression and the prevalence of the disease – regional lymphogenic metastasis.

3. Study of the lymphogenic prevalence (distant metastasis) of diffuse and intestinal gastric cancer on clinical examples.

4. Study of the features of Helicobacter pylori infection in patients with gastric cancer according to a rapid urease test.

**Object of study:**

Patients with diffuse and intestinal gastric cancer.

**Subject of study**

- single nucleotide mutations of the CDH1 gene
- protein E-cadherin
- metastatically affected lymph nodes
- Helicobacter pylori and gastric mucosa

**Research methods**

1. General clinical examination: filling in statistical cards, collecting data and anamnesis (detection of the presence of relatives of the first and second line of kinship with a diagnosis of stomach and breast cancer, age under 40), DNA sampling (venous blood sampling in vacutainers), isolation DNA, determination of DNA concentration, genotyping of germline mutations and SNPs.

2. Conducting an IHC study for the expression of E-cadherin, assessment of the level of expression;

3. Collection of laboratory and instrumental data from the examination of patients with diffuse and intestinal cancer of the stomach (computed tomography, ultrasound, magnetic resonance imaging), trephine biopsy, puncture aspiration biopsy of lymph nodes affected by metastases;

4. Determination of urease activity of a biopsy of the gastric mucosa (invitro) using a device for express diagnostics;

5. Quantitative, qualitative, information-analytical methods of statistical analysis.

**Scientific novelty**

- Data on molecular genetic aspects of diffuse and intestinal gastric cancer among the population of the Republic of Kazakhstan were obtained

- Distant lymphogenous metastases of gastric cancer were detected in operated patients that do not fall under the generally accepted classification.

- Data were obtained on the features of Helicobacter pylori infection in patients diagnosed with diffuse gastric cancer.

### **Practical significance**

A diagnostic method has been developed and applied, which makes it possible to detect genetic burden (TNF $\alpha$ , IL1B, IL10, TP53 and CDH1 genes, germinal mutations of the CDH1) in patients with gastric cancer;

The reduced expression of E-cadherin confirmed the possibility of predicting regional lymphogenous metastasis. It is necessary to take into account molecular markers for the formation of groups of poor prognosis (secondary prevention). It should also be taken into account that weak expression of E-cadherin (complete or no staining) is not necessarily accompanied by the presence of germline mutations in the CDH-1 gene in somatic cells. Therefore, an IHC study for the expression of E-cadherin in the tumor tissue for the purpose of a preliminary assessment of the presence of germinal mutations in the CDH-1 gene is not appropriate.

In the process of timely diagnosis of disease progression (metastases), in addition to ultrasound / CT of the OBP, X-ray CT of the chest, ultrasound of the OMT in patients operated on with a diagnosis of gastric cancer in the volume of gastrectomy with lymph node dissection, retrograde metastasis and metastases to the pelvic organs are possible: Krukenberg metastases (ovaries), metastases Schnitzler (lymph nodes along the iliac vessels), as well as a subsequent pattern - metastasis to the structures of the inguinal regions along the femoral vessels (inguinal lymph nodes), which necessitates MRI of the pelvic organs, ultrasound of the inguinal lymph nodes in this category of patients (tertiary prevention).

Taking into account the rather frequent occurrence of Helicobacter pylori infection in patients with diffuse gastric cancer, eradication of this infection should be recommended to first-line relatives (primary prevention).

### **Provisions for defense**

1. To develop and apply diagnostic methods to detect genetic burden (CDH 1 gene mutations) in patients with hereditary diffuse gastric cancer;
2. Low, weak or complete absence of E-cadherin glycoprotein expression increases the metastatic potential of the tumor in the lymph nodes;
3. In patients operated on for gastric cancer in the amount of gastrsplenectomy with D2 lymph node dissection, retrograde lymphogenous metastasis to the inguinal lymph nodes is possible;
4. The incidence of Helicobacter pylori in diffuse gastric cancer is higher and infects more parts of the stomach than in intestinal gastric cancer and in people who are not burdened with a malignant neoplasm of the stomach.

**Conclusions:** 1. Polymorphisms rs1042522 of the TP53 gene and rs1800896 of the IL10 gene are associated with the risk of developing stomach cancer, while polymorphisms rs1800872 and rs1800871 of the IL10 gene have protective properties against stomach cancer. The results of genotyping of the coding part of the CDH1 gene (exons) showed that no mutations were detected in the probands and in the family members included in the analysis.

2. Weak expression of E-cadherin (complete or no staining) and the depth of invasion in gastric cancer, potentiates the ability of tumor cells to lymphogenic

metastasis. There is a weak expression of E-cadherin in all studied cases of diffuse gastric cancer.

3. Cases of metastatic lesions of the testicle and inguinal lymph nodes in diffuse and intestinal gastric cancer that do not fit the generally accepted classification of distant lymphogenic metastases have been identified.

4. According to the correlation analysis, statistically significant results were obtained, which allow us to conclude that with diffuse RV with age

#### **Approbation of the dissertation**

The main results of the study and the provisions of the dissertation were reported and discussed at:

- X Congress of oncologists and radiologists of the CIS and Eurasian countries in memory of Academician N.N. Trapeznikov (Russia, Sochi, April 23-25, 2018);

- International Symposium BIOTECH 2018, June 12-13, 2018

- conference "Innovative technologies in medicine" (Samarkand, November 2018);

- International scientific and practical conference of students and young scientists dedicated to the 60th anniversary of NAO MUA - 2018 "(Nur-Sultan);

- XI Congress of oncologists and radiologists of the CIS and Eurasia named after N.N. Trapeznikova (Kazan, April 23-25, 2020)

- International scientific and practical conference of young scientists and students, dedicated to the 30th anniversary of the Independence of the Republic of Kazakhstan (Nur-Sultan, December 09-10, 2021);

#### **Publications on the topic of dissertation.**

13 scientific papers have been published on the topic of the work: 3 of them are in publications recommended by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan (Astanalyk medical journals, Valeology, Medicine), 5 - in the materials of international scientific and practical conferences; 3 - in publications indexed in the international information databases "Scopus" (Annals of Tropical Medicine, Electronic Journal of General Medicine, Bosnian journal of basic medical sciences); 1 - in the republican journal, 1 in "Scientific reports on clinical cases" (Acta Scientific Clinical Case Reports).

#### **Personal contribution of the dissertation student**

The dissertator independently conducted a survey of patients, sampling and DNA extraction, genotyping for CDH1 gene mutations, performed fine-needle aspiration biopsies, trephine biopsies of lymph nodes, took biopsies of the gastric mucosa and tested for urease activity of *Helicobacter pylori* with an express test system.

The dissertator independently and in a team analyzed and summarized the results of the study, carried out statistical data processing.

#### **The volume and structure of the dissertation work**

The dissertation work is presented on 116 pages of computer text, illustrated with 58 figures, contains 7 tables. Consists of an introduction, literature review, research materials and methods, experimental part, conclusions, list of references, including 150 titles, 4 appendices.