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Mutation Frequency in BRCA1 and BRCA2 Genes Among Women with Breast Cancer in Kabardino-Balkaria

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***Abstract* – The study was conducted within the individual genotyping of healthy and sick women in the Kabardino-Balkarian Republic to determine the risk-prone groups with increased susceptibility to hereditary form of breast cancer development. Blood samplings were collected and total DNA was taken from 300 people (control and experimental groups). The frequency of 5382insC, 4154delA, 185delAG BRCA1 mutations and 6174delT mutations of the BRCA2 gene among women with breast cancer was studied. The data obtained along with the survey suggest the hereditary or sporadic nature of the disease. A comparative analysis of the frequencies of genes alleles towards susceptibility to the hereditary form of this disease will provide an opportunity to discover the genetic reasons for an increase in cancer and to identify the groups of the greatest risk.**

Keywoords – breast cancer (BC) gene BRCA1, gene BRCA2, heriditary susceptibility, Kabardino-Balkaria.

I. INTRODUCTION

The study was conducted within the individual genotyping of healthy and sick women in the Kabardino-Balkarian Republic to determine risk-prone groups with increased susceptibility to hereditary form of breast cancer development. Blood samplings were collected and DNA of more than 300 people was taken (control and experimental group). The frequency of mutations of 5382insC, 4154delA, 185delAG of the BRCA1 gene and the mutation of 6174delT of BRCA2 gene in women with breast cancer was studied. A comparative analysis of the frequencies of genes alleles towards susceptibility to the hereditary form of this disease will provide an opportunity to discover the genetic reasons for an increase in cancer and to identify the groups of the greatest risk.

Breast cancer is one of the most common nosological forms within cancer diseases among women worldwide and in Russia. Molecular etiopathogenesis of malignant breast tumors is under intensive study. It has already been established that various hereditary mutations that maintain the integrity of genome (BRCA1, BRCA2, PTEN, TP53, CHEK2, MLH1, MSH2, LKB1 / STK11, CDH1) make a significant contribution to a disease development [1].

The development of new approaches in diagnosing and treatment of precancerous conditions is associated with the high level in malignancy rate.

The new instrumental methods to examine the female population allow not only to diagnose but also to correctly interpret the results. Hereditary breast cancer (BC) is the most common type of familial neoplastic disease. Its contribution to the overall number of breast cancer cases is approximately 5-10% [3]. A great resonance in society and among oncologists caused the discovery of BRCA1 gene in 1994, which in case of mutations is the cause of about half all cases of hereditary breast cancer [8]. A year later, the second gene (BRCA2) was discovered, which is also associated with a hereditary predisposition to breast cancer [7, 9]. In women with

mutations in these genes, breast cancer develops in 70-80% of cases, with a mutation in BRCA2 gene, a risk towards ovarian cancer increases [4].

Currently, the priority trend in the modern medicine is the early predictive diagnosis of diseases. The degree of success in treatment depends on the ability to identify a disease at the initial stage. In this regard, the development and introduction into medical practice of effective methods for predicting and early diagnosis using molecular genetics technologies is very topical. These technologies make it possible to identify mutations in the genes that control the cell cycle and the repair of DNA damage, which increase a risk of pathology to be studied.

The world is intensively studying the spectrum of mutations in the genes of BRCA family. This spectrum is very specific in different populations. Using DNA diagnostics and knowledge about the spectrum and mutation frequency specifics within a country or a region, it is possible to identify risk-prone groups of people having the increased susceptibility to oncopathology.

In Russia the conducted studies were targeted at examining the mutation spectrum of BRCA1 and BRCA2 gene among patients with familial forms of the disease in Moscow and Leningrad regions; the populations living in Bashkiria and Chita region were also involved and studied [2, 5]. The mutation 5382insC takes the leading position in the frequency of cases to happen. The results obtained are essential for practical DNA diagnostics towards the hereditary susceptibility to breast cancer and ovarian cancer in Russia, mainly among women belonging to the Russian ethnic group.

Published data about similar works and research in the North Caucasus are not available.

II. Methods and materials

The material to conduct the study was the DNA samples of healthy women and women with breast cancer; both groups are from Kabardino-Balkaria. Since 2009, the samples of venous blood have been collected on the basis of GBUZ Oncology Center of the Ministry of Health (Republic level) and Kabardino-Balkaria State University. The data have been collected via Oncology Dispensary; all of the data have been based on the analysis obtained from the comprehensive examination and treatment of 150 breast cancer patients aged from 25 to 78 years. In all cases, the diagnosis of breast cancer was verified morphologically. To date, the work continues on replenishing the DNA bank from a number of patients with breast cancer and healthy people. The blood samplings were collected within the consent obtained from patients who were informed about the study purpose. The population-genetic data of the examined healthy (control group) and breast cancer patients (experimental group) were obtained through survey approach.

DNA isolation from whole blood was performed with QIAamp DNA Blood Mini Kit reagent kit (Qiagen, Germany). Allele-specific PCR reaction was performed with PCR (polymerase chain reaction) reagent kit for identifying mutations 5382insC and 4154delA, 185delAG of BRCA1 gene and 6174delT of BRCA2 gene (Izogen company, Russia; Litekh company, Russia). PCR fragments were separated in 2 % agarose gel.

III. Results

Since, there is specificity in mutations distribution and in distribution of polymorphic variants of different genes among different nations, the genetics studies of various diseases should take into account an ethnic origin of the experimental and control groups. The study clarified the data within the ethnicity of patients (experimental group) through ; this helped to have the data about the nationality up to the third generation (parents, grandparents, grand-grandparents). In the control and experimental group three dominating nations can be distinguished: Kabardians, Balkarians and Russians.

In terms of age, the control and experimental groups differed. In the control group the age of women ranged from 20 to 69 years (average age was 31.3 years) (Fig. 1).

In the group with BC diagnosis the age spectrum was from 25–78 years old (the average age was 52 years old) (fig. 2).

When allocating the patients with BC along the age line, the peak in disease manifestation is fallen down on the age between 50 to 59 years old, which accounted to 40% of the entire number of the patients.

When collecting and processing clinical material, special attention was paid to identifying cancer cases in the families. Among the patients, there were noted the cases of various oncopathologies in the history of representatives within the different degrees of consanguinity (Table 1).

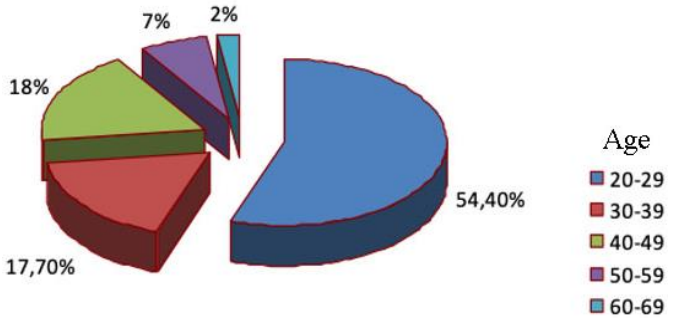

Fig. 1. Age spectrum in the control group

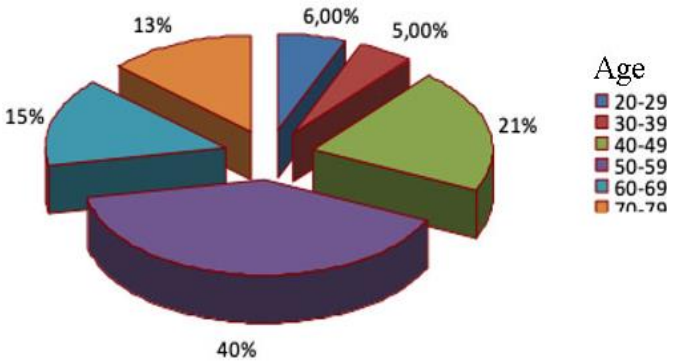

Fig. 2. Age spectrum of patients with BC (experimental group)

Many scientific studies resulted in the risk increase in appearing and developing breast cancer in early menarche and

late menopause. This risk is associated with impaired hormonal function in ovaries, adrenal glands, thyroid, pituitary, and hypothalamic system [6]. Among the studied group (experimental), the average age of menarche was 13.6 years old. The allocation among the patients according to the state of reproductive function is shown in Table 2.

TABLE I. RATIO BETWEEN BC AND OTHER ONCOLOGY DISEASES WITHIN THE SAMPLING TO BE STUDIED

Consanguinity	Oncopathology cases (%)	Family cases of BC (%)
I	13	6
II	6	2
III	2	1
Total	21	9

TABLE II. ALLOCATION PATIENTS WITH BC ALONG THE NATURE OF MENSTRUAL-OVARIAN FUNCTION

Nature of menstrual-ovarian function	Number of patients	
	Absolute number	*%*
Menses happen	82	54,6
Menopause	68	45,4
Total	150	100

TABLE III. ALLOCATION IN CONTROL GROUP ALONG THE NATURE OF MENSTRUAL-OVARIAN FUNCTION

Nature of menstrual-ovarian function	Number of representatives	
	Absolute number	*%*
Menses happen	107	71,3
Menopause	43	28,7
Total	150	100

TABLE IV. MUTATION-BASED CASES IN BRCA1 AND BRCA2 GENES

	Gene BRCA1			Gene BRCA2
	5382insC	*185delAG*	*4154delA*	*6174delT*
Patients with BC	4 (2.6%)	0	0	2(1,3%)
Healthy people (without BC)	0	0	0	0

90 % of the patients have children; 86 % of these women were breastfeeding. Due to the survey, we found that 98 % of the experimental group did not smoke, did not take hormonal contraceptives, 82 % had no work associated with the harmful effects from production processes, and only 18 % had work previously associated with chemical reagents and isotopes. Repeated (not once) early abortion was detected in 80 % of the women in the experimental group.

Among the control group, the average age of menarche is 12.8 years old. 61 % have children, 97 % of them were breastfeeding. The allocation among the women in the control group within the state of the reproductive sphere is shown in Table 3.

The clinical feature of breast cancer among the patients living in Kabardino-Balkaria is revealed, depending on two factors: the time when the disease was diagnosed and a woman's body state in terms of reproductive function.

The presence of mutations as 5382insC, 185delAG, 4154delA of BRCA1 gene and 6174delT of BRCA2 gene is investigated. The data presented in table 4.

Among the women (n = 150) patients with breast cancer, there is a high percentage of those who carry (2.6 %) of 5382insC mutation of BRCA1 gene; this mutation has been detected among 4 women. In these cases, an earlier age of manifestation of the disease among the carriers (women) of this mutation was noted. Two women had cases of the 6174delT mutation of BRCA2 gene (1.3 %). Mutations 185delAG, 4154delA in BRCA1 gene were not detected. When conducting genotyping, in the control group among healthy individuals, any mutations were not identified.

IV. CONCLUSION

The presence of the major mutation in BRCA1 gene (5382insC) allows screening studies to be carried out in high-risk groups and among women with uncomplicated family anamnesis with the purpose of timely prevention of this pathology (breast cancer).

The work over genotyping conducted in the control and experimental groups is going on with the aim to identify persons as carriers of other mutations.

The comparison in the frequencies of the alleles of the genes responsible for susceptibility to a hereditary form of breast cancer in both groups will allow to identify the genetic causes influenced the growth of cancer incidence, as well as to identify groups that can be referred to high risk category.

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