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HUMAN PAPILLOMAVIRUS AS A FACTOR IN THE DEVELOPMENT OF CERVICAL CANCER

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The analysis of cytological samples of 100 women aged 23 to 60 years was carried out by liquid-based cytology. The predominance of NILM in the samples in comparison with intraepithelial lesions of the cervix was noted. Among intraepithelial lesions, low-grade LSIL was significantly more common. Positive HPV tests were found in less than half of the cases in tested women. Among the positive tests of high oncogenic risk the types 16 and 51 were considered as high-risk HPV types. There is approximately equal positivity of the high-risk HPV test in women with NILM and intraepithelial lesions (LSIL, HSIL, ASCUS). The positivity of the high-risk HPV test depends on age: in women under 45 years this indicator is higher than in women 46 years and older.

According to our survey, with cytological study it is necessary to apply integrated test approach when screening high-risk HPV infection. The combined use of liquid-based cytology and HPV testing can improve the effectiveness of diagnostics by reducing the amount of uninformative material and allows to detect pathological changes at an earlier stage. It will significantly increase the value of diagnostic measures and will improve cytologist's experience in diagnosing and giving recommendations in clinical practice of cervical pathologies.

Keywords: human papillomavirus, cervical cancer, diagnostics, liquid-based cytology.

Cervical cancer is in the 2nd place among malignant neoplasms of female reproductive organs and 4th in the incidence structure of cancer among the female population, accounting for about 12% of malignant tumors in women and 60-80% of all forms of genital cancer [7]. Every year, 440 thousand new cases of cervical cancer are detected in the world, which is 5.8% of the total incidence of cancer [4]. Epidemiological and molecular-biological data indicate an important role of HPV in the occurrence of cervical intraepithelial neoplasia (CIN) and cervical cancer (CC) [5]. In the 70s herpes simplex virus serotype 2 (HSV-2) was suspected to be a carcinogenic agent. However, 20 years of experience in studying the role of this virus did not come to a positive conclusion [1]. Possibly, the virus associated with cytomegalovirus, bacteria, and protozoa can act as a co-factor of carcinogenesis, causing the development of dysplasia, and keeping it in a state of stabilization. Numerous papers published in 1980-1990 found that human papillomavirus (HPV) had links with dysplasia and squamous cell carcinoma of the cervix. Hybridization methods showed that 80 to 100% of cervical cancers contained DNA of HPV. A rough correlation was found between the frequency of CC and the detection of HPV in the population. For example, in countries with a high frequency of CC, the detection rate of HPV infection was about 10-20%, while in countries with a low frequency - 5-10% [10].

Characteristics of the aetiopathogenesis of squamous cell carcinoma in distal segments of female genitals are determined by external causes, where the viral infection is the most important factor. The causes of infection occurrence: decreasing the body defenses after infectious diseases, smoking, alcohol, young age, endometriosis, a large number of sexual partners, the presence of partners who had contact with a woman suffering from cervical cancer, anogenital condyloma, repeated abortions, inflammatory diseases, genital infections (chlamydia, trichomoniasis, genital herpes, etc.). The virus is 2 times more common in women who use oral contraceptives than in those who use barrier contraceptives or are postmenopausal [25].

It's been proven that HPV replication at the initial stage leads to an inflammatory reaction in the cervix, and later to the appearance of atypical cells in the thickness of the epithelial layer with varying disorder severity of differentiation, to changes in the layerage of epithelial cells. HPV-associated cervical dysplasia CIN develops [2]. According to C.M. Wheeler (2006) 3 years after HPV infection, one in four women has the progressing CIN II-III [32]. Human papillomavirus is a DNA-containing virus consisting of two structural genes (L1 and L2) encoding capsid proteins, and seven functional genes (E17) involved in HPV genome replication and transcription, cell cycle, signal transmission and control of apoptosis, immune modulation, and structural modification of the infected cell. More than 200 types of HPV are known, and more than 40 of them can affect the mucous membranes of the genitals [17]. HPV is divided into viruses of "high oncogenic risk" (16, 18, 31, 33, 35, 39, 45, 50, 51, 52, 56, 58, 59, 64, 68, 70) and "low oncogenic risk" (3, 6, 11,13, 32, 42, 43, 44, 72, 73). The "high-risk" viruses are more often detected in severe dysplasia, preinvasive and invasive carcinoma. In squamous cell carcinoma, type 16 HPV is most common (in more than 50% of cases), while type 18 HPV is more often associated with adenocarcinoma and low-grade cancer. The "low risk" viruses are identified with pointed and flat condyloma, mild dysplasia, and rarely with invasive cancer [9, 10].

The most dangerous for the development of a precancerous state – cervical epithelial dysplasia – is a long-term infection caused by type 16 HPV. Long-term HPV18 infection leads to the development of cancer in the endocervix. Infection with type 16 papillomavirus is common to find among all types of oncogenic HPV. It is found in more than 20% of women infected with papillomaviruses. As a comparison, HPV18 was found in 7% of the examined women with viral infection [34]. The epidemiological situation is showing the result of the tendency that has been emerging over the past twenty years. It is based on a steady increase in the frequency of infection caused by

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HPV16 with a constant percentage of infection associated with non-oncogenic types of HPV (HPV 6, HPV 11). This is all the more concerning since this infection affects young women aged 20-30-year-old. The incidence of HPV infection is the highest in the age group of 15-19-year-old: about 40% of positive tests indicate the presence of HPV DNA in material taken from the cervix. The percentage of positive results decreases with the increase of the surveyed women age: more than 30% among the 20-24 age group, and less than 30% among the 25-29 age group. The group of women under the age of 30 shows a further decrease in the frequency of HPV infections to 15-17% of the population. Papillomavirus infection is very rare in post-menopausal women but has an important prognostic value due to the risk of developing cervical pathology. Papillomavirus infection is very rare in post-menopausal women but has an important prognostic value due to the risk of developing cervical pathology. As a rule, human papillomavirus infection in postmenopausal women has the character of long-term persistent infection. This is very dangerous in terms of the occurrence of molecular changes leading to the initiation of the carcinogenesis process [35].

The peculiarity of HPV infection is the fact that epitheliotropy of the virus is not detected in the blood, and the production of antibodies by the immune system is not observed in all infected patients [31]. At the same time, the antibodies level is very low and is not able to provide long-term and reliable immunity. It can be explained by the ability of HPV to "escape" from the immune system of the macroorganism, which allows it to persist for a long time due to evolutionary acquired features (the replication cycle is limited by the epithelium, there is no viremia, cytolysis, peritransplant immunosuppression due to viral proteins) [19,40]. The persistence of highly oncogenic HPV types for more than two years is the most dangerous factor in the progression of precancerous cervical cancer. When HPV type 16 persists, the risk of developing cervical intraepithelial neoplasia (CIN) is 40-50%; when HPV type 26 is 30-40%; HPV type 31, 58, 82 is 20-30%; HPV type 18, 33, 35, 51, 52 is 10-20% [32].

The target for papillomavirus exposure is the transformation zone, the area of contact between the multilayer flat and prismatic epithelium of the cervix. It is the place of development of precancerous conditions. It was found that up to 94% of papillomas are located in the transformation zone or distal [14]. Cervical damage caused by HPV, in the form of cervical intraepithelial neoplasia (CIN), can be mild, moderate, or severe. Women with low-grade CIN are at risk for severe CIN and cervical cancer. However, CIN 1 does not always progress to CIN 2-3. The age of women with cervical intraepithelial neoplasia is usually 5-10 years lower than with grade of CIN1, which indicates a gradual progression of changes in the cervical epithelium [41]. Probably the oncogenic types of HPV infection, which affects the cervix, are determining the risk of severe cervical intraepithelial neoplasia in women [29]. The frequency of detection of HPV DNA increases as the degree of dysplasia worsens and the transition of dysplastic epithelium changes into the tumor. HPV DNA in CIN 2 is detected 24-25%, in CIN 3 - 61-80%, and in microinvasive carcinoma - 83-88% [28, 30]. In invasive carcinoma and carcinoma in situ, HPV DNA was detected in more than 90% of cases. Papillomaviruses are known to belong to the family Papovaviridae. The genome is a double-stranded circular DNA, and reproduction occurs in the nucleus of host cells. The human papillomavirus, like many DNA viruses (herpes simplex, Epstein-Barr virus, etc.), is a biological agent that can modify the growth, differentiation, and morphology of cells. When a virus enters cell, it causes changes in its structural, biochemical, and genetic organization, and introduces foreign genetic information into it. The formation of binuclear and polynuclear cells is a characteristic feature of the cytodestructive action of the virus. The formation of binuclear cells is usually based on the fusion mechanism of plasma membranes of two cells; a number of viruses contain enzymes that can lyse cell membranes [29]. The formation of intranuclear and cytoplasmic inclusions, vacuoles is the main characteristic of many DNA viruses. It is possible that the mechanism of vacuolated cell formation is based on the water and energy metabolism process of cells. The formation dynamics of inclusions, their shape, size, and content of nucleic acids and proteins in them are of great diagnostic importance since they differ in various groups of viruses. The cytoplasm of cells accumulates oxyphilic masses containing viral protein and RNA, numerous viral particles that form vacuoles. Chromosome pulverization, karyolysis, karyorrhexis, pyknosis develop in the nuclei of cells; nuclei move to the periphery. Under the cytopathogenic action of viruses, cell destruction is directly or indirectly related to the penetration of the virus genome and its functioning. The resulting changes in cell morphology may be the result of one or more factors: inhibition of the synthesis of cellular DNA, RNA, and proteins [43]. Infection with the virus is a necessary but insufficient condition for the development of malignant neoplasms. Along with this, there are additional factors, including genetic ones, involved in the transformation of normal cells [31,37,39]. Two genes present in the HPV genome cause malignant cell transformation [18]. These viral oncogenes are named E6 and E7. The E6 and E7 genes are multifunctional. The ability to activate cellular telomerase is the one of the mechanisms of their action. Telomerase is an important enzyme for maintaining the stability of chromosome telomeres during multiple cycles of cellular proliferation. Increased telomerase levels are observed in severe intraepithelial injuries [13, 27].

Another point of influence of the E6 and E7 genes is the suppressor proteins p53 and Rb (retinoblastoma gene), which induce apoptosis. The effect of the virus is manifested in the inactivation of suppressor proteins and inhibition of apoptosis. Increased expression of the p53 gene in CIN I, compared with more marked injuries, indicates its protective role in the process of carcinogenesis and may be one of the diagnostic criteria for damage assessment and disease progression [38,44] However, the expression of E6, E7 genes is not enough to cause not only proliferation but also the transformation of cells. The "High-risk" HPV invades the host cell to induce chromosomal aberrations and gene mutations. The accumulation of cell mutations with a latently persistent virus subsequently serves as an endogenous factor in the progression of tumor cells [23].

Cervical carcinogenesis initiated by human papillomaviruses can be divided into several stages [4]: 1) primary infection with the virus; 2) persistence of the human papillomavirus genome in the episomal form and the capability of viral particles production with subsequent secondary infection; 3) integration of viral DNA into the cell genome without visible specificity of the integration site; at II and III stages, the functions of E6 and E7 begin to manifest, violating the control of cell division; 4) induction of mutations in cell DNA, causing instability of the cell genome; 5) selection of a clone of cells with mutant DNA containing integrated DNA of human papillomaviruses; 6) active reproduction of this clone of cells and tumor growth. This mechanism explains the fact that from the moment of viral infection to the appearance of a tumor takes a long time – 5-10 years.

Diagnostics of HPV-associated diseases of the cervix should be complex and based on several studies: detection of HPV DNA using a real-time polymerase chain reaction with typing and determination of the number of genomic equivalents of the virus; enzyme immunoassay (ELISA) - an immunological method for the qualitative or quantitative determination of E7 HPV 16 and 18 type cancer proteins based on a specific antigen-antibody reaction; cytological examination (traditional or liquid-based); expression assessment of P16/Ki67 oncoproteins by immunocytochemical (ICC) and immunohistochemical (IHC) methods; extended colposcopy; histological examination of a cervical biopsy [3].

The main methods for diagnosing HPV include polymerase chain reaction (PCR), which allows genotyping and quantification of 21 type of HPV. The method is based on an amplification reaction, during which primer molecules bind to fragments of DNA virus in a sample and in with the addition of catalysts, and under the influence of temperature, daughter complementary double-stranded DNA molecules are formed [12]. According to several researchers, high-grade dysplasia and CC determination needs to conduct a cytological study in combination with HPV testing, the sensitivity reaches up to 96% [33Confirmation of the etiological role of human papillomavirus (HPV) in the development of CC has now led to HPV testing being considered as a possible component of screening for this disease. It is important to separate HPV infection and HPV-associated disease (precancer) and to determine the risk of its progression. Практически любые методы выявления ДНК ВПЧ обладают 95–100 %-ной диагностической чувствительностью по отношению к тяжелым дисплазиям и раку шейки матки [6]. Almost all methods for detecting HPV DNA have a 95-100% diagnostic sensitivity to severe dysplasia and cervical cancer [6]. But the qualitative determination of HPV DNA has controversial clinical significance, since it does not allow predicting the course of infection. It is considered that a negative HPV test means the patient has a low risk of developing cervical cancer in the next 3 years. However, if the HPV test is positive, this does not mean that the patient has a high risk of developing cervical cancer. Primary cytological examination of the cervix and cervical canal is a classic screening method for detecting changes in the cervical epithelium [8, 11, 22]. Despite the exceptional value of cytological examination of cervical smears for the preinvasive form diagnosis of the tumor process, the frequency of false-negative results of the test can reach 50% in the invasive form diagnosis of CC [10, 24, 26, 36, 45].

In recent years, the method of liquid-based cytology (LBC) has been actively introduced in addition to traditional cytology, which contributes to ensuring the quality of sampling, storage, and transportation of material [12]. An important technological feature of the LBC method that improves the quality of the study is that the test material is placed in a special stabilizing solution during standardized sampling, which ensures its safety without changing its morphological and immunocytochemical properties. According to generalized data, the sensitivity of the traditional method was less than 60%, and the LBC method was 95 %. The specificity of the methods was 40 % and 66%, respectively. The correlation with the results of the histological examination does not exceed 60 % by the traditional cytological method; it is close to 100 % (99 %) by the LBC method [20]. The authors of the research concluded that the LBC method is a more reliable laboratory test: reduces the number of false negative results, reduces the number of unsatisfactory drugs for analysis, and reduces the time required for a cytologist to evaluate cell material. In addition, the same sample taken for LBC can be used for detection of HPV of high oncogenic risk and determination of ICC using CIN cancer markers [4].

In recent years, one of the most promising methods for the diagnosis of HPV-associated cervical lesions is immunocytochemical analysis. The determination of proliferation markers P16 and Ki-67 expression can determine not only the presence of viral proteins in the cell but also the degree of violations of cellular regulation in response to the persistence of the virus in the cell. The atypical squamous cells cervix expressing both P16 and Ki-67 indicates induced cell cycle dysregulation and provides a more accurate diagnosis of high-severity lesions. The first studies on the determination of P16 and Ki-67 showed high specificity of the method [12]. The combined use of LBC and immunocytochemical analysis of the tumor marker p16ipk4a in the screening of CC will allow detecting tumors at the early stages of development, and at the stages preceding it when the curability of this disease is close to 100% [16].

An additional examination method of patients with cervical pathology is extended colposcopy. According to S. I. Rogovskaya, the sensitivity of colposcopy for determining subclinical human papillomavirus infection, precancerous cancer, and breast cancer is 80-90%, and the specificity is 30-60% [15]. The advantages of this method include the possibility of targeted biopsy from the most suspicious areas of the cervix for further histological examination. However, it is known that even if the diagnosis of dysplasia is morphologically verified, the probability of dysplasia turning into cancer is less than 50 % [42].

Therefore, confirmation of the causation of HPV in cervical cancer development has led to the fact that the diagnosis of papillomavirus infection, along with cytological studies, has become considered an essential element of screening and prevention of this disease. At the present stage of the occurrence of cervical cancer, it is safe to assume that long-term infection with HPV 16 and 18 is the most important risk factor for the development of this tumor. The success of new diagnostic methods will lead to early detection of the atypical transformation of cervical epithelial cells, and risk assessment factors for tumor transformation. Modern methods of the diagnosis and treatment of papillomavirus infection would open up new opportunities for understanding and controlling malignant pathology of the reproductive system in women caused by papillomavirus infection.

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MUTATIONS IN BRCA1/2 GENES IN PATIENTS OF SOUTHERN RUSSIA WITH MALIGNANT OVARIAN TUMORS

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The article presents the results of a study of the spectrum of mutations in the BRCA1 / 2 genes associated with the development of hereditary breast and ovarian cancer in patients of South Russia with malignant ovarian neoplasms.

Mutations in the BRCA1 gene were determined by real-time PCR: 185delAG, 300T> C, 2080delA, 4153delA, 5382insC, 3819delGTAAA, 3875delGTCT; in the BRCA2 gene - 6174delT in 178 patients with a histologically verified diagnosis of ovarian cancer.

The study included epithelial tumors (malignant) - 98.1%, and granulosa cell tumors - 1.9%. Of the epithelial tumors, the most common was high-grade serous carcinoma (78%). Based on the results of genotyping, the prevalence of germline mutations in the BRCA1 / 2 genes was revealed at 20.8%. The higher rate of genetic changes is obviously associated with hereditary history (40% of patients). Of the seven identified mutations, 5382insC (67.6%) was revealed more frequently. All patients confirmed the same mutation in the tumor. There were no cases of somatic changes in BRCA1 / 2. The prevalence of BRCA1 mutations was noted in the group of patients with low-grade serous carcinoma, in which all cases of mutations in the BRCA2 gene were identified.

Thus, in patients with OC living in the south of Russia, the mutation frequency in the BRCA1 / 2 genes was 20.8%. The distribution of mutation types with predominance of 5382insC BRCA1 (67.6%) corresponds to the ratio of their occurrence in populations of European countries. BRCA1 / 2 mutations were recorded more frequently in the group of patients with high-grade serous carcinoma.

Keywords: ovarian cancer, mutations, BRCA1 / 2, PCR.

Introduction. Ovarian cancer (OC) is a malignant tumor and the first leading cause of death among gynecologic cancers due to a large number of patients with advanced disease characterized by metastases into the abdominal cavity. About 10-15% of ovarian tumors are associated with hereditary diseases, and about 65-85% of patients with hereditary ovarian carcinomas have a mutation in the BRCA1 and BRCA2 genes involved in DNA repairing and genomic stability maintenance [16]. The lifetime risk of OC developing in women with mutations in the BRCA1 or BRCA2 gene is 35-70% and 10-30%, respectively [16]. The total lifetime risk of developing breast cancer and OC increases to 85% and 60%, respectively, in carriers of BRCA1 and BRCA2 mutations [8]. Hereditary OC was reported to be associated with the presence of gene aberrations related to other hereditary syndromes (TP53 mutations in Li-Fraumeni syndrome, mismatch repair genes (MMR) in Lynch syndrome, double-strand break repair genes BARD1, CHEK2, RAD51, and PALB2) [17].

Aim of the study. The aim of the study was to analyze the spectrum of mutations in the BRCA1/2 genes associated with the development of hereditary breast cancer and ovarian cancer in patients with ovarian cancer in the South of Russia.

Material and methods. The study included 178 women aged 27-71 years old diagnosed with ovarian cancer T1-4N0-1M0-1, gr. 2 (stage I-IV), who received treatment in National Medical Research Centre for Oncology in 2015-2019. The survey revealed one or more risk factors in 71 cases: the age of onset up to 45 years, multiple tumors, bilateral lesions, and hereditary burden. All patients gave their informed consent to the processing of their personal and medical data, as well as to the use of biological material. EDTA venous blood and paraffin-embedded ovarian tissue blocks were studied. Genomic DNA was isolated from whole blood leukocytes and tumor samples using the DNA-sorb-B kit (AmpliSens, Russia). For DNA isolation from paraffin blocks (tissue samples fixed in 10% buffered formalin), 5-8 slices 3 μm thick were obtained using a microtome, dewaxed with o-xylene and 95% ethyl alcohol, lysed overnight in 200 μl of lysis solution with the addition of 20 μl of

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