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## DETECTION OF HUMAN PAPILLOMAVIRUS OF HIGH CARCINOGENIC RISK IN WOMEN WITHIN THE ONCOSEARCH PROGRAM IN YAKUTIA

The study was conducted as part of a pilot project at the Yakut Republican Oncological Dispensary titled ОНКОПОИСКСАХА.РФ (ONCO-SEARCH), which is part of the national healthcare project. In 2021, 724 women from 5 uluses (villages) of the Republic of Sakha (Yakutia) and the city of Yakutsk took part in the study. The overall infection rate of high carcinogenic risk human papillomavirus (HCR HPV) among women in the Zhigansky Ulus was 10.6%, the Verkhoyansky Ulus – 4.7%, the Churapchinsky Ulus – 11%, the Namsky Ulus – 8.3%, the Srednekolymsky Ulus – 10.9%, and the city of Yakutsk – 5.5%. HCR HPV genotypes 16, 18 and 45 were identified as the most aggressive forms integrated into the human genome. In the article, we present the prevalence of HPV types by districts. We also highlight differences in infection among women of different age groups. The largest proportion of HCR HPV carriers falls on the age groups of 20-30 years (14.6%) and over 70 years (13.9%).

**Keywords:** human papillomavirus, screening, integrated forms, age distribution.

**Introduction.** Human papillomavirus (HPV) is one of the most common infections in the world, which is divided into categories of low and high carcinogenic risk. HPV causes skin and anogenital warts, neoplasms of the oropharynx, cervix, anal canal, vulva, vagina, and penis [4, 7, 8, 10]. Harald zur Hausen and his colleagues were first to demonstrate that

genital warts contain the human papillomavirus genome [9, 11]. Later, the main cause of cervical cancer (CC), as well as precancerous lesions, was attributed to HPV infection (certain types), transmitted mainly through sexual contact. In 2008, German scientist Harald zur Hausen was awarded a Nobel Prize for discovering the role of HPV as a cause of cervical cancer.

According to 2020 data, cervical cancer takes fourth place (after breast, colorectal, and lung cancer) in global cancer incidence among women of all age groups. In Russia, according to data for 2018, cervical cancer accounted for 5.3% of all oncological conditions in women [1]. At the same time, in terms of prevalence related to the reproductive period in the age group of 15 to 44 years, it ranks second. Regarding geographic distribution, data are presented on the highest prevalence of HPV in developing countries in low- and middle-income regions. In Eswatini (Africa), this figure is 84.5 cases per 100 thousand people; Bolivia (South America) – 36.6; Maldives (Asia) – 24.5; Montenegro (Europe) – 26.2; Fiji (Oceania) – 29.8. In Europe, Russia ranks 13th with 14.1 cases per 100,000 people.

According to the WHO concept [2], the fundamental ways to combat cervical cancer include vaccination, screening, diagnosis, and treatment of precancerous conditions and invasive cervical cancer. Diagnosis at an early stage of cervical cancer and immediate treatment in most cases can avoid the progression of the disease and contribute to recovery. Cervical screening programs based on a cytological examination (Pap test) and/or a molecular diagnostic PCR method

[6] make it possible to identify women at high risk of developing cervical cancer and prevent the disease in a timely manner. Statistics show that among all age groups, the prevalence of HPV averages about 20-30%. However, different studies show highly variable results between regions and social groups, which indicates the focal nature of the spread of HPV [3]. At the same time, the published data on HCR HPV in Russia and Yakutia, in particular, are based to a greater extent on studies of patients seeking medical help, which does not allow an objective assessment of the overall prevalence of the virus among the population.

**The Purpose of the Study:** mass screening testing, and analyzing carriage and characteristics of the viral load of 14 oncogenic HPV types in women living in various uluses (villages) of the Republic of Sakha (Yakutia).

**Material and Methods.** Women participated in the voluntary screening study (n = 724), they were from 5 uluses (villages) of the Republic of Sakha (Yakutia) and the city of Yakutsk, aged 20 to 86 years. The mean age was 50.6±8.6 years. The study materials were epithelial cells of scrapings from the cervical canal, placed in a transport medium for liquid cytology with volumes of 2-5 ml. DNA extraction was carried out with the AmpliSens® DNA-sorb-D reagent kit designed for isolating total DNA from epithelial cells taken for liquid cytology. For amplification and detection of HPV DNA segments (multiplex PCR), we used the AmpliSens® HCR HPV screen-titer-14-FL test system, which makes it possible to identify the 14 most potent oncogenic types of high carcinogenic risk using one fluorescent channel with ROX dye:

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- for genotypes 16, 31, 33, 35, 52, 58 – region E1 gene
- for genotypes 18, 39, 45, 56, 59, 66, 68 – region E2 gene
- for genotype 51 – region E7 gene

Separate channels allowed us to detect the 16th (FAM), 18th (JOE), and 45th (Cy 5.5) genotypes in the E6 gene regions (Table 1). Detections of the E6 region with no detections in E1/E2 regions point to the integration of the virus into the human genome.

Additionally, we used the AmpliSens® HRC HPV genotype-titer-FL reagent kit. Amplification was carried out on a 6-channel detecting amplifier with a Real-time CFX-96 Touch 96 x 0.2 ml thermal block from Bio-Rad (USA). Amplification was set up and the results were analyzed using the FRT Manager (version 3.4). Statistical data processing was carried out using Statistica 6.0.

**Results and Discussion.** Some studies have shown that the most common HCR HPV genotypes, in descending order of occurrence, are: 16, 18, 59, 45, 31, 33, 52, 58, 35, 39, 51, 66, and 68. However, we also note variability in genotypic

prevalence depending on the geographical region. Knowledge gained about the prevalence and genotype structure of HPV in a particular geographic region can be of great help in choosing a strategy to prevent HPV-associated diseases. As a result of our studies, we found that the total infection rate with HCR human papillomavirus among all examined women ( $n = 726$ ) was 7.6% ( $n = 55$ ; Table 2). In the structure of the studied districts of the Republic of Sakha (Yakutia), the highest infection rates were noted in: Churap-chinsky Uls – 11.0%, Srednekolymsky Uls – 10.9%, Zhigansky Uls – 10.6%, and Nam-sky Uls – 8.3%. In the city of Yakutsk and the Verkhoyansk Uls, these figures were 5.5 and 4.7%, respectively. It should be noted that in Zhigansky and Churapchinsky Ulses, there have been cases of simultaneous carriage of two HCR HPV genotypes, and three in Srednekolymsky Uls (Table 2). We registered combinations of genotypes 16 with 18, as well as 16 with 31 and 56, respectively. It is also noteworthy that out of the total number of screened women in the Verkhoyansk Uls, there have been infections with only two HPV genotypes, 16 and 18.

The integration of the virus into the host cell's genome plays a special role in the development of HPV diseases; likewise, the development of cervical cancer is often associated with the integration of virus DNA into the genome [5]. Most often, HPV 16 and 18 genotypes are integrated, while the E1/E2 section breaks apart while maintaining the E6/E7 oncogene.

The viral genes E6 and E7 play a key role in the process of tumor transformation. Their activity is controlled by the upstream regulatory region (URR). Oncoprotein E6 plays the role of a coactivator that interacts with transcription factors and elements of the main transcription complex. Thus, the E6 gene is a multifunctional protein. Its transactivating activity involves regulation of transcription, interaction with the p53 gene and its degradation, and violations in the cell growth control mechanism and in the process of cell differentiation. The E7 protein is able to reverse cell arrest in the G1 phase of the cell cycle, exert a mitogenic effect, and stimulate uncontrolled DNA synthesis [9, 12]. Thus, cervical cancer is a unique model of carcinogenesis associ-

Table 1

Distribution of Fluorophores by Detection Channels

Channel for Fluorophore	FAM	JOE	ROX	Cy5	Cy5.5
Target DNA	HCR HPV DNA Genotype 16	HCR HPV DNA Genotype 18	Genotypes 16,18,31,33,35, 39,45,51,52,56, 58,59,66,68	Plot DNA $\beta$ -Globin Gene (Internal Control Sample Glob)	HCR HPV DNA Genotype 45
Amplification Region	E6 gene	E6 gene	E1 Gene (for genotypes 16, 31, 33, 35, 52, 58)/ E2 gene (for genotypes 18, 39, 45, 56, 59, 66, 68)/ E7 gene (for genotype 51)	$\beta$ -Globin Gene	E6 Gene

Table 2

Structure of HCR HPV Infection

Uls (Village)	HPV 16 <sup>1</sup>	HPV 18 <sup>1</sup>	HPV 45 <sup>1</sup>	HCR <sup>2</sup>	Mixed (Geno-types), Persons	Negative, Persons	Total, Persons/(%)	Infected, Per-sons/(%)
Zhigansky	5	-	2	5	1 <sup>1</sup> (16.18)	110	123/(17.0)	13/(10.6)
Verkhoyansk	3	1	-	-	-	81	85/(11.7)	4/(4.7)
Churapchinsky	-	1	2	5	1 <sup>1</sup> (16.18)	73	82/(11.3)	9/(11.0)
Namsky	1	-	-	5	-	66	72/(10.0)	6/(8.3)
Srednekolymsky	1	1	-	3	1 <sup>2</sup> (16.31.56)	49	55/(7.6)	6/(10.9)
Yakutsk	2	4	2	9	-	290	307/(42.4)	17/(5.5)
<b>Total:</b>	<b>12</b>	<b>7</b>	<b>6</b>	<b>27</b>	<b>3</b>	<b>669</b>	<b>724/(100)</b>	<b>55/(7.6)</b>

<sup>1</sup> – genotypes –region E6 gene (likely integrated into the human genome);

<sup>2</sup> –genotypes 16, 31, 33, 35, 52, 58 – E1 gene region, genotypes 18, 39, 45, 56, 59, 66, 68 – E2 gene region, geno-type 51 – E7 gene region.

ated with a viral infection, its uniqueness is due to the exogenous genetic information with trans-forming potential in tumor cells.

Figure 1 shows the distribution of HCR HPV forms integrated into the genome against the background of general infection with the virus in the uluses of the Republic of Sakha (Yaku-tia). The average value for the studied regions of the Republic was 3.9%. In the Verkhoyansk Ulus, despite the low overall infection rate (4.7%), all forms of the virus were integrated. We identified three cases of the 16th genotype and one of the 18th genotype of the E6 gene region, while other genotypes of the E1 gene/E2 gene/E7 gene regions were not identified (Table 2).

In the Zhiganskyy Ulus, 8 cases (6.5%) out of 13 (10.6%) infected were integrated forms of HPV 16 and HPV 45, with a predominance of the 16th genotype ( $n = 5$ ). In one case, we identified the integration of two genotypes at once: 16 and 18. In Churapchinsky, we noted genotypes 18 and 45 (1 and 2 cases, respectively) and, just like in the Zhiganskyy Ulus, one case of an integrated form of two genotypes at once – HPV 16 and HPV 18. We identified only one case of carriage of the integrated form of HPV 16 in the Namsky Ulus. In the Srednekolymsky Ulus, we recorded one case of a non-integrated form of HPV 16 carriage. We detected the E6 gene region of HPV 16 and the HRC E1/E2/E7 gene region. This may indicate that the integration of the virus into the human genome has not yet occurred. In this case, a more effective response to therapeutic measures with a more favorable prognosis is likely. We also highlight one case of carriage of integrated forms of 16 and 18 genotypes. In Yakutsk, 8 out of 17 cases had integrated forms: 2 cases each for genotypes 16 and 45, and 4 cases for HPV 18. We have not detected HCR HPV 51 genotype with the E7 gene detection region.

To identify the age characteristics of HPV distribution, the subjects were divided into 6 age groups: 20-30 years (5.7%), 31-40 (16.9%), 41-50 (27.3%), 51-60 (24.4%), 61-70 (20.6%), and older than 71 (5.1%; Fig. 2).

Analysis of the distribution of HCR HPV showed that the largest proportion of carriers were in the groups of women aged 20-30 (14.6%) and over 70 (13.9%) (Fig. 3). The lowest infection was observed in the age group of 41-50 and amounted to 2.0%. It should be noted that in our study, the age samples of younger and older women were relatively small ( $n = 41$  and  $n = 36$ , respectively).

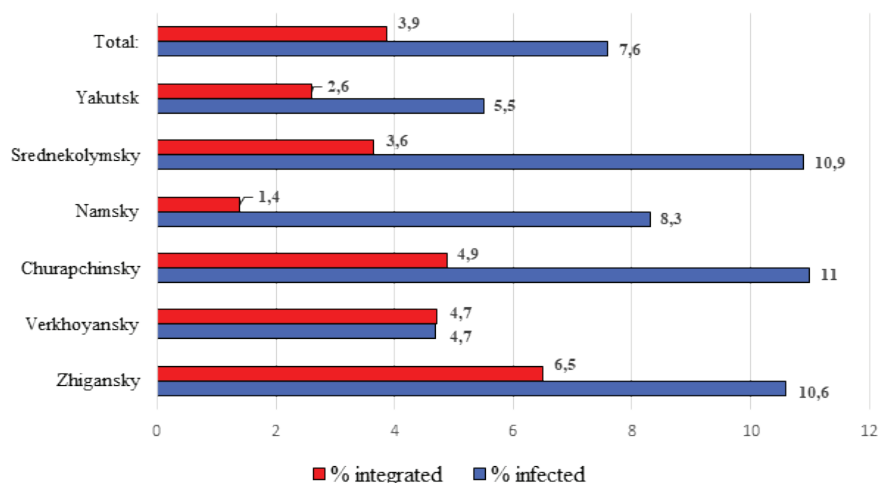


Fig. 1. Structure of the Territorial Distribution by Uluses of General Infection and HPV Forms Integrated into the Human Genome (%)

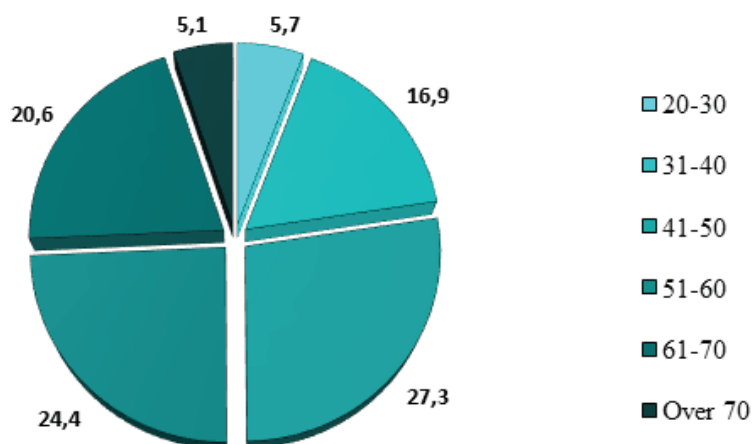


Fig. 2. Structure of the Distribution of the Studied by Age Groups (%)

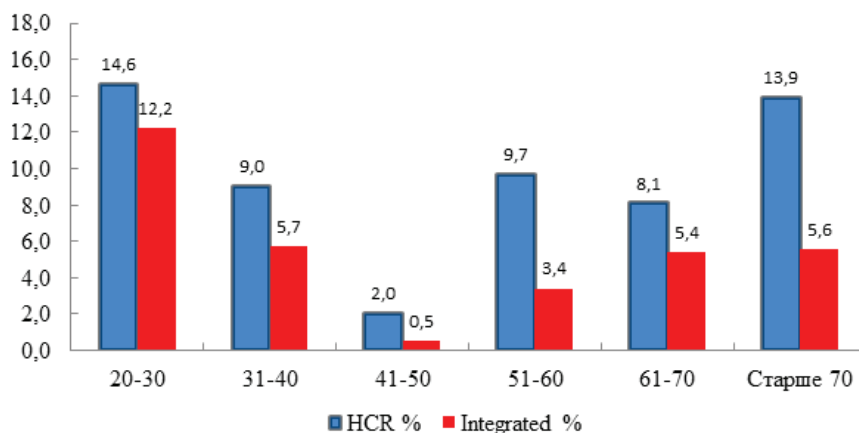


Fig. 3 Distribution of HCR HPV and Integrated Forms of the Virus by Age Groups X-Axis: Age (Years); Y-Axis: Infection (%)

Therefore, in order to obtain the most reliable statistical data on the age characteristics of HPV distribution in the Republic, it is necessary to involve more women of this age.

The maximum proportion of integrated forms of HCR HPV was found in the group of women aged 20-30 (12.2%) and 31-40 (5.7%). Then, in decreasing order, there were groups of: older than 70 (5.6%), 61-70 (5.4%), 51-60 (3.4%) and the smallest number of cases (0.5%) of integrated forms of the virus was found in women aged 41-50.

With this study, data on the spread of HCR HPV in the regions of the Republic, and especially integrated forms of the virus, have been obtained for the first time. Further research is needed to obtain more accurate statistics.

**Conclusion.** Thus, we have established that the overall infection rate in the Republic varies from 5.5% (Yakutsk) to 11% and averages 7.6%. The highest overall infection rate was found in the Zhiganskyy Ulus – 10.6%, the Churapchinsky Ulus – 10.9%. In the same uluses, cases of infection with two or more HCR HPV genotypes were noted. We detected integrated forms of HPV were detected in 3.9% of women, and non-integrated forms in 3.7% of cases. The structure of integrated forms is dominated by the HPV 16 genotype (1.7%), followed by HPV 18 (1.0%) and HPV 45 (0.8%). The largest proportion of HCR HPV carriers

falls on the age groups of 20-30 years (14.6%) and over 70 years (13.9%). The largest proportion of the forms of the virus integrated into the human genome was found in women aged 20-30 and 31-40 years – 12.2% and 5.7%, respectively. The smallest number of cases (0.5%) of integrated forms of the virus was observed in the group with a low percentage of virus carriers (2.0%) among women aged 41 to 50 years.

All detected cases of infection have HPV genotypes of high oncogenic risk and, with a high probability, can cause cervical cancer and severe dysplasia. All the patients with HCR HPV infection were registered and directed for further research (Pap test and colposcopy), in accordance with the cervical screening program in the conditions of the Yakut Republican Oncological Dispensary under the ОНКОПОИСКСАХА.РФ (ONCO-SEARCH) pilot project.

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