



## Effect of Hyperestrogenemia on the Development of Cervical Intraepithelial Neoplasia in the Case of Human Papillomavirus

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### ABSTRACT

The article describes the study of the hormonal status of the human papillomavirus (HPV) carrying patients with the developed low-severity squamous intraepithelial lesion (LSIL). The aim of the study was to identify the relationship between elevated serum estradiol levels and the development of mild cervical intraepithelial neoplasia (CIN I). The number of analyzed samples included in the control and experimental groups was determined. Cytological preparations of cervical scraping were prepared and stained by the automated BD PrepStain system. The genotyping of HPV was carried out by sampling the material of the cervical canal. The hormonal status of patients in three age groups was analyzed. The results of the cytological examination, PCR, and hormonal analysis of the groups were compared with each other. In most patients of the experimental group, an increase in serum estradiol, prolactin, cortisol, and FSH was noted. It was also revealed that the majority of patients with elevated levels of serum estradiol had cervical intraepithelial neoplasia (CIN I). It was experimentally established that the percentage of dysplasia development with elevated estradiol levels has similar values in the case of HPV-16, HPV-18, and other genotypes.

**Keywords:** Human papillomavirus (HPV), Low-grade squamous intraepithelial lesion (LSIL), Cervical intraepithelial neoplasia (CIN I), Hyperestrogenemia.

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### INTRODUCTION

Currently, human papillomavirus (HPV) is one of the most common infections worldwide. Persons of reproductive age, in particular, young women aged 18-35, are at higher risks [1]. The risk of infection is associated with a high risk of developing cervical cancer [2]. As a rule, in this pathology, HPV WRC is detected in 99.7 % of cases [3]. The risk of infection increases in the absence of barrier contraception, features of sexual behavior, or the presence of concomitant infections. The virus's contagiousness is quite high – 80% with a single sexual contact [4]. About 660 million people worldwide are

infected with HPV. About 30 million cases of CIN I and about 10 million cases of CIN II-III are diagnosed annually [5-7]. According to Russian studies, in some regions, the HPV incidence rate is 120-150 per 100 thousand people, and the national average is 20 per 100 thousand people. According to the WHO, about 500 thousand cases of cervical cancer are reported annually in the world [8].

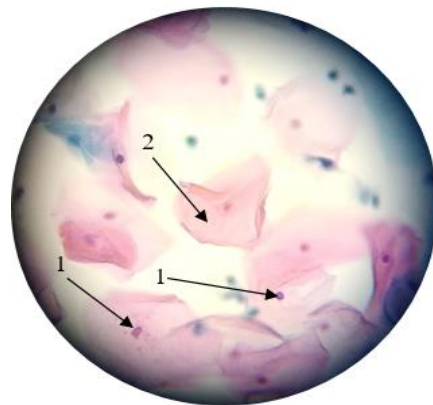
The human papillomavirus causes damage to the mucous membranes of the upper respiratory tract, rectum, and genitals. Morphologically, cellular proliferation, which is accompanied by the replication of the viral genome, manifests itself in the form of warts and papillomas, as

well as the appearance of coilocytosis. The flat and glandular epithelium of the cervix is affected by HPV of high carcinogenic risk. The transformation zone is the most vulnerable. Viral DNA is embedded in the DNA of cells and the expression of the E6 and E7 genes increases. The products of these genes reduce the effect of the tumor suppressor protein p53 and retinoblastoma protein pRb. Normally, p53 is responsible for initiating the replication of the cell's DNA as it controls the phases of its cycle and blocks tumor processes. The expression of the E6 gene leads to the synthesis and accumulation of the E6 protein, which, in combination with the E6-AR protein, participates in the degradation of p53 and reduces its amount in the cell. In the future, this leads to cell immortalization [9]. The pRb protein blocks the replication process when DNA damage is detected. The total action determines the transition of the cell from the G<sub>1</sub>-phase to the S-phase. There is an E2F kinase that provides this transition. Normally, it is in a complex with pRb, so it is inactive. The product of the E7 gene - the p16ink4 $\alpha$  protein - separates this complex. It is also involved in the methylation of suppressor genes, which leads to their instability. Cell proliferation increases but the cells do not reach maturity. The protein p16ink4 $\alpha$  is an early marker of the precancerous condition of the cervix, so its detection is of great diagnostic importance [10].

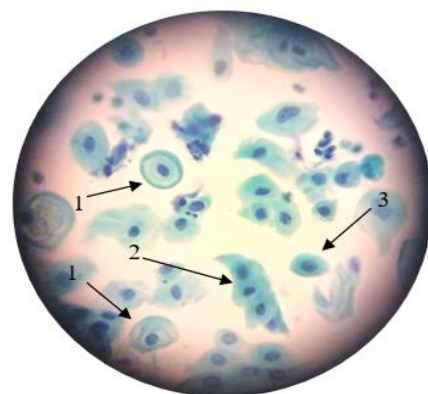
The development of cervical intraepithelial neoplasia in the case of human papillomavirus is influenced by many factors. According to some authors, hormonal imbalance can provoke the phenomenon of cervical neoplasia [11]. In our work, we evaluated the effect of elevated estradiol levels on the development of CIN I. As is known, estradiol (E<sub>2</sub>) is converted to 16 $\alpha$ -hydroxy estrone (16 $\alpha$ -ONEE1) and 2-hydroxy estrone (2-ONEE1) as a result of metabolic reactions. The first has an increased carcinogenic effect as it binds very strongly to estrogen receptors through covalent bonds. 2-ONEE1, on the contrary, normalizes cell growth. 16 $\alpha$ -hydroxy estrone stimulates the synthesis of the oncoprotein E7, thereby accelerating the process of tumor transformation [12].

## MATERIALS AND METHODS

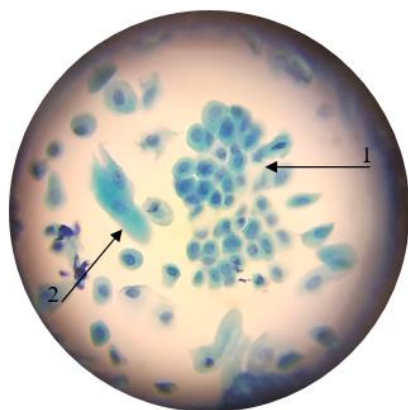
The study was conducted in the clinical and diagnostic laboratory of the Stavropol Regional Clinical Hospital in Stavropol City. We examined 28 patients with the result of cytological examination of cervical scraping "LSIL" (**Figure 2**) and "LSIL CIN I" (**Figure 3**), (according to the Bethesda classification), and 23 patients with a satisfactory result - NILM (control group), (**Figure 1**). In all patients, the presence of HPV was confirmed by PCR. Patients infected with HPV of several genotypes, representing different carcinogenic risks, belonged to the group of the most oncogenic type.



**Figure 1.** Surface cells of squamous epithelium with small nuclei and abundant cytoplasm. They are located mostly scattered. The result is NILM. 1. Cell nuclei. 2. Cytoplasm. The preparation was made by the method of liquid cytology, stained according to Papanicolaou. x400



**Figure 2.** The lesion of the squamous epithelium of a low grade. HPV. The result is LSIL. 1 - Koilocytes - intermediate cells of stratified squamous epithelium, hyperchromic nucleus, pronounced perinuclear clearing zone. 2 - Dyskeratocytes - cells of squamous epithelium, polygonal elongated, with hyperchromic nuclei and dense cytoplasm. 3 - Parabasal cell. The preparation was made by the method of liquid cytology, stained according to Papanicolaou. x400



**Figure 3.** Dysplasia of the cervical epithelium of low grade. The result is LSIL CIN I. 1 - a layer of parabasal cells. 2 - Atypical cells of irregular, elongated shape, with signs of dyskeratosis, the cytoplasm is intensely colored. The preparation was made by the method of liquid cytology, stained according to Papanicolaou. x400

Cytological preparations were made and stained by Papanicolaou using the automated BD PrepStain system. The liquid method of preparation is preferable to the traditional one since the endocervix and ectocervix cells are included into the test sample. Also, the quality of the preparation by liquid cytology method is much higher than that prepared by a laboratory assistant or a gynecologist on their own.

In order to determine the relationship between the development of mild cervical neoplasia (CIN I) and hormonal status, an immunochemical analysis of blood serum was performed in 28 patients with the result of cytological studies "LSIL" and "LSIL CIN I" and confirmed HPV. In 23 patients of the control group, the result of carrying HPV was confirmed by PCR and hormonal status studies were also conducted. In

the study, all patients were divided into three age categories. In women of reproductive age, the study was conducted on days 4-7 of the menstrual cycle.

Statistical data analysis was carried out by the method of variation statistics using "Microsoft Excel 7.0".

## RESULTS AND DISCUSSION

The study of the hormonal status, according to some researchers, can determine the prognosis of the development of certain pathologies. Estrogens can influence the development of cervical neoplasia since this pathology develops in the transformation zone – the area of the junction of the multilayer flat epithelium of the vaginal part of the cervix and the glandular epithelium of the cervical canal.

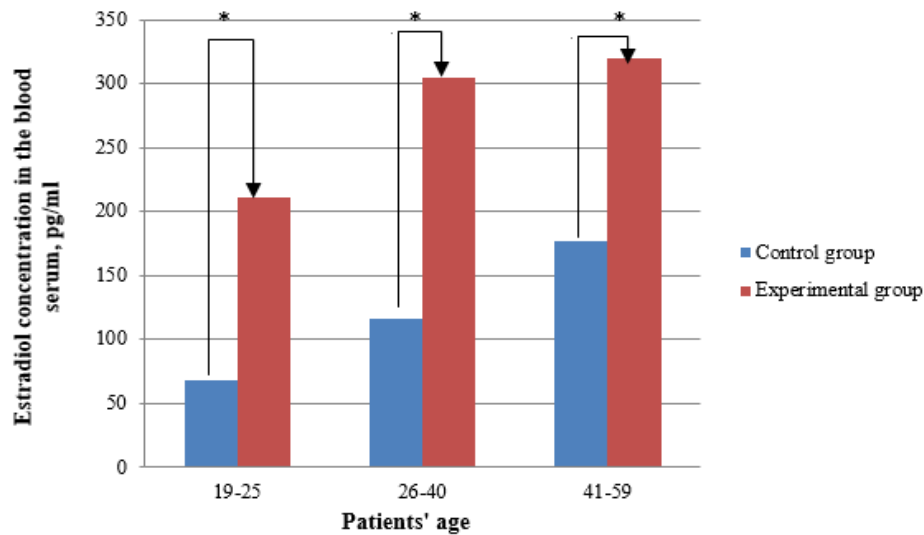
When studying the hormonal status of the experimental group of patients, it was revealed that all 28 had deviations according to the results of the study. Of the 23 patients in the control group, 12 had normal serum hormone levels. When summarizing the data obtained, it was revealed that among the patients of the control group, hyperestrogenemia was observed in 21.7% of cases, in 26.1% there was an increase in other hormones, and in 52.2 % of cases, the result of the hormonal study was normal.

Among the 28 patients of the experimental group, all results of the study of hormonal status had deviations. In 79% of cases, there was a significant increase in estradiol, and in 21% of cases, there was an increase in other hormones.

**Table 1.** The level of estradiol in the blood serum of patients of different age categories in the control and experimental groups

|                    | Level of estradiol in the blood serum |                    |                    |
|--------------------|---------------------------------------|--------------------|--------------------|
|                    | 19-25 years old                       | 26-40 years old    | 41-59 years old    |
| Control group      | 68.243 ± 16.628*                      | 116.382 ± 64.165*  | 177.393 ± 25.856*  |
| Experimental group | 210.678 ± 23.032*                     | 304.796 ± 121.784* | 319.135 ± 101.292* |

Note: \* indicates the significance of the differences between the values  $p \leq 0.05$

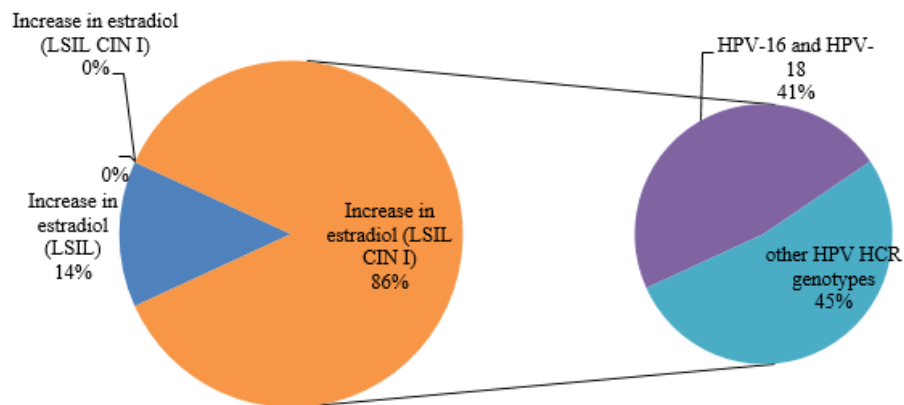


**Figure 4.** The level of estradiol in the blood serum of patients of different age categories in the control and experimental groups

Note: \* indicates the significance of the differences between the values  $p \leq 0.05$

In women aged 19-25, an increase in serum estradiol by 3 times was noted ( $p < 0.05$ ) in comparison with the control group. In patients aged 26-40, an increase of 2.6 times ( $p < 0.05$ ) was observed in the estradiol level compared with the control group. In the age group between 41 and 59, the increase in estradiol was 1.8 times ( $p < 0.05$ ) in comparison with the control one (**Table 1 and Figure 4**). According to the cytological study, 19 patients of the

experimental group with elevated estradiol levels had a mild degree of dysplasia (CIN I). As it is known, HPV-16 and HPV-18 play the greatest role in the occurrence of cervical neoplasia and breast cancer. However, according to the results of the PCR study, out of 86% of patients with hyperestrogenemia and CIN I only 41% had the most carcinogenic genotypes (**Figure 5**).



**Figure 5.** Number of cases of HPV-16, HPV-18, and other genotypes of the virus in hyperestrogenemia and the presence of CIN I

**CONCLUSION**

The study of the endocrine profile in women can help determine the prognosis of the course of certain pathologies. Estrogens, in particular estradiol and its metabolites, contribute to the

occurrence of hyperplastic processes in the female reproductive organs. These factors can be considered from the perspective of synergy. Hyperestrogenemia is a favorable factor for the development of HPV-associated cervical intraepithelial neoplasia. In our study, 86% of

patients with elevated estradiol levels in case of HPV had cervical intraepithelial neoplasia. It should be noted that out of 86% of cases of hyperestrogenemia and "LSIL CIN I", the most carcinogenic HPV genotypes – 16 and 18 – were found in 41%. It means that estrogens can influence the development of CIN in the case of the human papillomavirus.

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