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Anthropometric Characteristics of Female Patients with HPV-Associated Cervical Squamous Cell Lesions of Low Degree Depending on the Expression of Oncoprotein P16

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A B S T R A C T

Along with well-known factors, significantly significant risk factors for the progression of HPV-associated squamous cell cervical lesions include: constitutional affiliation with the microsomal and micromesosomal somatotypes, a family history of cervical cancer and severe intraepithelial neoplasia, the presence of the chlamydial antigen HSP-60IgG in the blood, chronic inflammation in the endo- and exocervix, multiple cervical retention cysts larger than 10 mm, a history of and current menstrual irregularities, the presence of extragenital pathologies (gastrointestinal and respiratory), and poor adherence to barrier contraceptive methods.

Introduction

The cervix, due to its structural and functional features, occupies a special place in the reproductive system, largely determining the prospects for full fertilization, uncomplicated pregnancy and timely delivery (1, 3). Cervical cancer ranks third in terms of incidence. In 2012, a twofold increase in the pathology was detected in women under 30 years of age. Every year, 370,000 new cases of cervical cancer are registered worldwide, which annually takes the lives of 190,000 women (2, 4, 6). At the same time, the increase in the frequency of unjustified surgical interventions on the cervix in women of reproductive age confirms the need for additional research in this area of gynecology (4, 8, 10). According to the published "Strategy for the Development of Medical Science in Uzbekistan until 2030" within the framework of the scientific platform "Reproductive Health", one of the priority areas is the development of screening programs for the detection of cervical diseases, taking into account regional characteristics (5, 9, 11). The primary role in the prevention of cancer and precancerous diseases is assigned to primary and secondary prevention and early diagnosis. These two areas are entirely within the competence of the primary obstetric and gynecological service (1, 12). According to

Kattakhodjaeva M. Kh. (2021), cytological screening exists in Uzbekistan, which can be called opportunistic (when visiting a gynecologist for another reason). Cervical screening should be based on a population registry, cover the majority of 5 vulnerable representatives of the population, and include active recall of patients for examination. The need for additional research in this section of gynecology is confirmed by the large proportion - up to 30.0% - of surgical interventions, sometimes repeated with the aim of eliminating the pathological process in the cervix (2, 14). The reason for such radicalism lies, apparently, in the lack of clear criteria for the choice of medical tactics, which should be substantiated not only by the visual picture of the pathology, but also by objective indicators of the expression of specific biomarkers (1, 13). The relevance of this problem is also confirmed by the fact that the detection of high-risk human papillomavirus infection has proven to be a method with insufficient specificity, especially in patients of early reproductive age due to the potential peak of primary HPV infection. This necessitates the introduction of new, effective, prognostically significant molecular tumor markers, including the cyclin kinase inhibitor p16ink4a. The safe clinical capabilities of immunocytochemical detection of this marker make it a promising method for monitoring cervical pathology in pregnant women. The study of the diagnostic value of outpatient use of modern diagnostic technologies, such as cyclin-dependent kinase inhibitors, p16ink4α protein (5, 14), which is the final clinical product of carcinogenesis compared to E6 and E7 proteins, the development of algorithms for the management of patients with cervical pathology, as well as the formation of risk groups subject to mandatory vaccination against the human papillomavirus within the framework of specialized reception rooms for cervical pathology - the path to the implementation of the National Screening System. Currently, there is evidence in the literature that diseases of a multifactorial nature, which account for 90-95% of all diseases, which include cervical intraepithelial neoplasia, are associated with body type features (6, 15). Numerous studies have been devoted to determining the relationship between somatotype, in particular with obstetric and gynecological diseases such as polycystic ovary syndrome (1, 16), preeclampsia (17), hyperandrogenism (18), and endometrial hyperplasia (1). According to many authors (2), immune system disorders that occur in various diseases may be associated not only with the possible damaging effects of external factors, such as human papillomavirus in cervical diseases, but also with a hereditary predisposition and defects in biochemical reactions. Somatotype is a clinical manifestation of the constitution and a genetically determined feature that determines the similarity of disease course options and their progression (2). These circumstances are especially important for diseases that resolve spontaneously and progress only in a small number of patients. There are several somatotyping techniques, each individual (3). Using these methods, it is possible to predict the course of the disease in an individual, prescribe personalized therapy, and identify risk groups available for primary prevention before the onset of clinical manifestations and even before infection with infectious agents. Data on the relationship between somatotypological characteristics and the patient's ethnicity and geographic location is increasingly being confirmed (4).

For many years, the Rostov Anatomical School has proposed using the modified Dorokhov-Petrukhin method (2022) in clinical trials due to its broad coverage of constitutional characteristics, accessibility and ease of implementation, the ability to automatically calculate somatotypes using computer applications, and its sufficient validation across various nosologies for clinicians. Data on the somatotypological characteristics of patients with cervical diseases, particularly mild cervical intraepithelial neoplasia, their management strategies, and prognosis for the course of the disease depending on the identified somatotype are lacking in the available literature.

Therefore, research into the clinical, anamnestic, and somatotypological characteristics of HPV-associated cervical diseases that predispose to progression is of scientific and practical interest.

The present study aimed to improve the clinical management of patients with mild cervical squamous intraepithelial neoplasia (CIN I) through a comprehensive prognostic approach integrating constitutional, somatic, and immunocytochemical investigations. The study focused on evaluating the combined diagnostic and prognostic significance of these parameters to facilitate early detection, accurate risk assessment, and effective therapeutic decision-making in patients with mild cervical epithelial abnormalities.

Materials and Research Method

The study utilized materials from annual market reports

from 2023 to 2025 from the Bukhara Region Perinatal Center's gynecology department, as well as statistical reporting forms from medical institutions performing cervical smear cytology using traditional methods and liquid-based cytology as part of screening under the compulsory medical insurance program. A total of 235 patients, aged 18 to 35 years, residing in Bukhara and surrounding areas of the Bukhara Region, were recruited for the clinical study. They were diagnosed with mild cervical intraepithelial neoplasia associated with various types of HPV, and had no abnormal colposcopic findings.

All patients underwent basic diagnostic methods for the condition of the cervix, including the traditional cytological method, its modification (liquid cytological analysis); HPV testing and colposcopy; examination for sexually transmitted infections using the PCR method; ELISA method for determining HSP-60 IgG Ch. trachomatis; oncoprotein p16ink4α.

All patients underwent basic cervical diagnostic tests, including traditional cytology and its modification (liquid-based cytology); HPV testing and colposcopy; screening for sexually transmitted infections using PCR; ELISA testing for HSP-60 IgG Ch. trachomatis; and cytological testing for the p16ink4α oncoprotein, a prognostic and diagnostic marker of disease progression.

Twenty-five women were diagnosed with mild cervical intraepithelial neoplasia associated with various types of HPV, without abnormal colposcopic findings. The results of cytological screening, HPV testing, colposcopy, and immunocytochemical determination of molecular tumor marker expression after 12 months of follow-up determined the distribution of the 235 patients into Group 1 and Group 2. Group 1 included 60 HPV-positive patients (mean age 27.4 ± 5.1 years) with mild cervical lesions accompanied by tumor marker expression. Colposcopic examination of these patients revealed mild lesions, such as thin acetowhite epithelium, delicate mosaicism, puncturation, or a combination of these. Group 2 included 175 patients (mean age 26.2 ± 6.4 years) with LSIL but no expression of the p16ink4α oncoprotein.

60 apparently healthy women, who were negative for HPV-associated cervical lesions based on cytological screening at their initial visit, constituted the control group. In the clinical groups, information on social status, menstrual function, contraceptive behavior, reproductive history, history of gynecological and extragenital diseases, previous surgeries, and cofactors for the development of cervical epithelial dysplasia were analyzed.

Cytological examination of smears from the vaginal portion of the cervix and cervical canal was performed using two methods: cytological examination of traditional preparations (PAP test) and examination of thin-layer preparations using liquid-based cytology. When preparing a traditional preparation, a cytobrush (such as a Cervix Brush) was inserted into the external os of the cervix, carefully guiding the central portion of the brush along the axis of the cervical canal. The cytobrush was then rotated 360° (up to 3-4 times clockwise), thereby collecting a sufficient number of cells from the ectocervix and transformation zone. The cytobrush was then removed and the sample was spread on a slide. The sample was transferred to a glass slide quickly, without drying or losing any mucus or cells adhered to the instrument. When preparing a thin-layer preparation using liquid-based cytology, after collecting the sample, the brush head (Combi-Brush) was detached from the handle and placed in a container with a stabilizing solution.

Slides prepared using the traditional method were stained using the Romanovsky-Giemsa method after thorough drying and fixation of the cellular material with 96° ethyl alcohol for 15 minutes.

Criteria for papillomavirus infection included the presence of koilocytic atypia in the cytological material, as well as acanthosis, parakeratosis, hyperkeratosis, and varying degrees of cervical intraepithelial neoplasia. Immunocytochemical determination of the expression of the molecular tumor marker, the cyclin kinase inhibitor p16ink4α, was performed from cervical epithelial samples obtained by liquid-based cytology with the CINtec© diagnostic kit in combination with rat antibodies.

Somatotype assessment was performed using a modified method by R.N. Dorokhov and V.G. Petrukhin (2017), which is based on a comprehensive metric assessment of morphological features across three main levels of variation: dimensional (characterizing body size); component (assessing the expression of fat, muscle, and bone components); and proportional (characterizing body proportions).

Study results and discussion. The body mass index

(BMI) was assessed based on body length and weight. Five main somatic types were identified: nano- (NaS), micro- (MiS), meso- (MeS), macro- (MaS), and megalosomal (MegS). A more in-depth assessment of constitutional features revealed two additional or transitional somatotypes: micromesosomal (MiMeS) and mesomacrosomal (MeMaS).

To clarify the role of somatotype—the morphological expression of constitutional type—in predisposing to the development of cervical HPV-associated neoplasms, an analysis of the dimensional characteristics, the degree of expression, and the nature of the relationships between the main anatomical components of the soma (bone, muscle, and fat), and body proportions was conducted in HPV-positive patients with LSIL, compared with these parameters typical of otherwise healthy women. It should be noted that immunocytochemical testing of the p16ink4α tumor marker in HPV-positive patients with LSIL revealed different disease progressions. Some women expressed the tumor marker (Group 1), while others had a negative reaction (Group 2). It was hypothesized that these changes may be characteristic of women with general biochemical abnormalities, including possible immune response disorders necessary for HPV persistence, its more rapid onset of carcinogenesis associated with hereditary factors, and clinically expressed by a specific somatotype.

Analysis of the mean values of DT and MT in patients expressing the biochemical marker of disease progression (Group 1) revealed that significant differences in body size were observed only when compared to apparently healthy women (the control group). Thus, among patients with p16ink4α (Group 1), the mesosomal type was 3.7 times less common than in the control group, while the microsomal and micromesosomal types were 7 and 3 times more common, respectively, and were observed in more than every second woman. In Group 2, patients without p16ink4α expression, the frequency of mesosomal, microsomal, and micromesosomal somatotypes was comparable to the control group.

In studying the component level of variation (CLV), we assessed the degree of development and the nature of the relationships between the main tissue components of the soma in patients with different variants of the course of low-grade cervical intraepithelial lesions (Groups 1 and 2) compared to the control group. A characteristic feature of the soma fat distribution in the majority of HPV-positive patients with disease progression to more severe lesions (Group 1) was a high frequency of "very low" and "low" body fat percentages (51.7% and 30%, respectively). More than half of the patients in Group 2 and apparently healthy women had a fat mass distribution of 53.7% and 56.7%, respectively.

Results of assessing

The muscle mass distribution in Groups 1 and 2 patients compared to apparently healthy women (the control group) revealed that the majority of patients in Groups 1 and 2 had "below average" muscle mass (43.3% and 63.4%, respectively), in contrast to apparently healthy women, in whom the predominant degree of this parameter (66.7%) was "average." Among patients in Groups 1 and 2, those with "average" muscle mass levels accounted for significantly lower proportions: 23.3% and 18.3%, respectively ($p<0.05$).

Analysis of the obtained data revealed that "very low" muscle mass levels were not observed in either study group. Among patients with disease progression (Group 1), "low" and "below average" muscle mass levels were significantly more common ($p<0.05$) compared to Group 2. "Average" muscle mass levels were predominant among control women and patients lacking p16ink4α. The distribution of other muscle mass levels across the study groups did not show significant differences.

A study of the proportional characteristics of women in the study groups revealed no statistically significant differences. Thus, in all groups (1st, 2nd, and the control group), representatives of the MiMeMb somatotype predominated (66.7%, 70.3%, and 58.3%, respectively). The obtained data characterized the regional characteristics of the studied cohort of women and the dependence of their somatotype according to the PUV on geographic latitude.

Thus, a comparative study of the somatotypological characteristics of women in the control group and patients with different variants of the course of low-grade cervical intraepithelial neoplasia (Groups 1 and 2) depending on the expression of the p16ink4α oncoprotein in the cervix allowed for a more complete presentation of the somatotypological characteristics. Microsomal and micromesosomal somatotypes, the relationships between the main components of body mass, characterized by "low" and "below average" content of LM, CM, along with the identified risk factors

(Chapter III), should be considered as morphological cofactors of predisposition to the development of HPV-associated low-grade squamous cell lesions. Statistically significant differences between somatometric parameters defining somatotype based on the dimensional and component variation levels of features in patients depending on the expression of the p16ink4α oncoprotein (Groups 1 and 2) allow us to understand their interrelationships and provide a comprehensive morphological, visual characteristic of the "risk group" for adverse disease development.

In conclusion, Determination of p16ink4α tumor marker expression in cervical smears with simultaneous HPV testing is indicated for women aged 18 to 35 years with micro- and micromesosomatic somatotypes based on the dimensional variation level of features. Personalized management strategies for mild HPV-associated squamous cell cervical lesions based on the developed mathematical model stratify patients based on the risk of disease progression and improve the quality of diagnostics.

Author Contributions

M. T. Khamdamova: Investigation, formal analysis, writing—original draft. Sh. Sh. Khamidova: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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