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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ
ТБИЛИСИ - НЬЮ-ЙОРК

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.) since 1994. **GMN** carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

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3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

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3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრაფიების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფხიხლებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

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10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

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THE INFLUENCE OF IMMUNODEFICIENCY ON THE LEVEL OF CD34-POSITIVE CELLS IN THE CERVIX

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Cervical pathology is widespread in the population, which is associated with low detection, imperfection of the medical system, sometimes with asymptomatic course or insignificant symptoms of the course of inflammatory and non-inflammatory processes in this area [1,2] despite real achievement in prevention of invasive carcinoma during last years. Although regular checkups and cytological screenings are designed to reduce morbidity and mortality rates among women, the problem of pathological processes in cervix is extremely acute today [3]. The prevalence of this group of diseases is still high both in Ukraine and worldwide [2].

Particular attention of medical personnel in terms of the complexity of the clinical course, diagnostic features, individual approach to treatment is paid to risk groups [4]. This group of diseases is characterized by the greatest variability and complexity of the course in women with immunosuppression [5,6]. At the same time, HIV-infected women and women suffering from alcoholism represent a global problem [7,8].

By reinforcing each other's effects, both HIV infection and changes caused by alcohol abuse trigger a chain of pathological reactions leading to reversible and often irreversible pathological processes in the cervix [9].

Ethanol is known to be the main component of all alcoholic beverages, belonging to category 1 carcinogens, which means that there are reliable signs of its carcinogenicity to humans. On the other hand, alcohol abuse lead to immunodeficiency with local and general decrease in immunoresistance invariably, which results in a violation of the physiological microbiome of the cervix, and therefore leads to an increased risk of infection with pathogenic microorganisms, fungal flora, and viruses, in particular, the human papillomavirus (HPV) [3].

To date, there are many studies on changes in cervix in alcoholism, as well as in HIV infection, however, there are no data about property of cervical tissue to keep potential for regeneration under influence of immunodeficiency, which is very important since it allows to escape severe irreversible changes in tissue. CD34 stem cells are one of significant indicator for property of tissual regeneration [10].

Considering all of the above, the purpose of our study was to identify amount of CD34 stem cells in the cervix under influence of immunodeficiency of infectious and non-infectious origin.

Materials and methods. For the study, sectional material was taken from 80 women of reproductive age from 20 to 40 years. All subjects were divided into 3 groups. The first group (25 women) included women with confirmed HIV infection without any data on concomitant alcoholism. In patients of the second group (25), only history and post-mortem signs of alcohol abuse were identified. In this group, alcohol abuse was confirmed both by history data (survey of relatives) and autopsy results (alcoholic cirrhosis of the liver as the main symptom). The

control group comprised women (30) who died from diseases not related to alcohol abuse, reproductive disorders without concomitant HIV infection (who died as a result of accidents or incidents).

The collected material was fixed in 10% neutral buffered formalin and then embedded in paraffin. At the next stage, 5 x 10-6 m thick sections were made from the prepared paraffin blocks. Sections were subsequently stained with hematoxylin and eosin. Immunohistochemical examination (IHC) was performed indirect immunoperoxidase reaction [11] with monoclonal antibodies (mAb) to CD34 (Thermo scientific, USA). The reaction was visualized using a set of UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen (Thermo scientific, USA). Microscopic examination was carried out on an Olympus BX41 microscope with further morphometric examination using the Olympus DP-soft 3.12 software.

The following indicators were determined: the thickness of the stratified squamous nonkeratinized epithelium (SSNE) in all groups of patients, the relative volumes of genital warts, expressed as a percentage. Assessment of the degree of infiltration of the mucosal lamina propria (MLP) by immunocompetent cells (lymphohistiocytes) using a semi-quantitative method from 0 to 3 points (0 – no, 1 – weak, 2 – moderate, 3 – severe infiltration). Semi quantitative assessment of the IHC-staining [12] was done using the following grading: 0 (up to 10% cells immunoreactive), +1 (>10% and up to 50% cells immunoreactive), +2 (>50% cells strongly immunoreactive).

Statistical processing of the results obtained was performed using the methods of variation statistics. The correspondence of the distribution to the normal one was determined by the Shapiro-Wilk's test, which showed that the samples were close to the normal distribution. Statistical indicators are presented as $M \pm \sigma$, where M is the arithmetic mean, σ is the standard deviation, Student's t-test. Correlation analysis was carried out using Spearman's rank correlation coefficient. The statistical difference between the studied parameters was considered significant at $p < 0.05$.

All studies were carried out in accordance with the Declaration of Helsinki, approved by the ethics commission of Odesa National Medical University (protocol 3, dated 17 October 2011).

Results. The study yielded findings, indicating the changes of the cervix in both investigated groups. The thickness of the epithelial layer of the mucous membrane of the cervix was determined. Table 1 presents the figures obtained when determining the thickness of the epithelial layer. The maximum thickness of the stratified squamous nonkeratinized epithelium of $714.23 \pm 59.21 \times 10^{-6}$ m was typical for the group of HIV-infected women. In the group of surveyed women who suffered from chronic alcoholism, this indicator was $695.7 \pm 47.27 \times 10^{-6}$

6 m. The expected minimum indicator was in the comparison group, where it amounted to $523.21 \pm 44.48 \times 10^{-6} \text{m}$. There was a statistically significant difference of 36.4% between the comparison group of HIV-infected women and the control group.

Table 1. The thickness of the cervical epithelium.

Indicator	Investigated groups		
	HIV infection	Alcohol abuse	Control group
Thickness of the stratified squamous nonkeratinized epithelium, 10^{-6}m	$714.23 \pm 59.21^*$	$695.7 \pm 47.27^*$	523.21 ± 44.48

* $p < 0.05$ significant between investigated groups and group of control.

One of the possible reasons for changes in the thickness of the epithelium may be a significantly more frequent development of condylomatous vegetations in the study groups, especially often associated with HIV infection. Histological examination of the cervix morphologically verified three forms of condylomatous vegetations were: pointed, flat and inverted. The relative volumes of distribution of such forms are presented in Table 2.

Table 2. Relative volumes of condylomas of the studied groups, % (n) among selected cases.

Types of condiloma	Investigated groups		
	HIV infection	Alcohol abuse	Control group
Pointed, %(n)	16,00 (4)	8,00(2)	3,33 (1)
Flat, %(n)	12,00 (3)	8,00 (2)	3,33 (1)
Inverted, %(n)	8,00 (2)	4,00 (1)	3,33 (1)
Total, %(n)	36,00(9)	20,00 (5)	10,00 (3)

The group of examined women with a combination of HIV infection (see Table 2) were found to have a greater number of different types of condylomas: genital warts in this group accounted for 16%, flat – 12%, inverted – 8 %, which in total amounted to 36%. It should be noted that in the group of women who abused alcohol, there was also an increase in the relative volume of genital warts among all selected cases. These figures were 8%, 8%, and 4% (20% in total) for pointed genital warts, flat warts, and inverted warts, respectively.

Other possible changes in the thickness of the epithelium can include much more frequent cases of cervical dysplasia. So, in most cases of the comparison group, there were no signs of dysplasia of the cervical epithelium, while in the studied groups, a significant increase in the specific volume of cases with dysplastic changes was detected. Low-grade squamous intraepithelial lesion (mild dysplasia) is characterized by preserved anisomorphism and stratification of the surface and intermediate layers, focal basal cell hyperactivity with an increase in the nuclear-cytoplasmic ratio. At the same time, the structuring of the nucleoli in the nuclei of the cells of the basal and parabasal layers, their moderate basophilia, is revealed.

Depending on the severity of dysplasia, the studied cervixes were characterized by normal, weakly, moderately or strongly

pronounced impaired cytoarchitectonics. The nuclei were of normal size or slightly enlarged, had the same oval shape, with predominantly normal polarization and a mild degree of hyperchromia. Mitotic figures were present in 85% of cases in all layers, in 10% they were observed only in the basal layer, in 5% they were few both in the basal and other layers of the epithelium.

In case of high-grade squamous intraepithelial lesion (high-grade cervical dysplasia), a pronounced violation of cytoarchitectonics was observed (the size and shape of the cells varied significantly). Cells with transparent cytoplasm, as a rule, were absent, or their small number was observed in some areas, the size of the nuclei varied markedly with a predominance towards increase, the nuclei had different shapes, there was no polarization in many, and there was a pronounced hyperchromia of the nuclei with the presence of even singular pathological mitoses and mitotic figures. The distribution of the studied material depending on the severity of cervical dysplasia is presented in Table 3. A high degree of distribution of cervical dysplasia severity was determined in the study material of HIV-infected patients, it comprised 12.00%.

Table 3. Distribution of cervical dysplasia severity (%) among selected cases.

Cervical dysplasia severity	Investigated groups		
	HIV infection	Alcohol abuse	Control group
Low-grade squamous intraepithelial lesion	32.00(8)*	28.00(7)*	13.33(4)
High-grade squamous intraepithelial lesion	12.00(3)*	8.00(2)	-

* $p < 0.05$ significant between investigated groups and group of control.

Assessment of the degree of infiltration of the mucosal lamina propria (table 4) showed uneven distribution for different group. It should be noted that in this group, 4.7% of cases were found to have no infiltration in HIV infection group, i.e. almost complete absence of lymphocytes. Evaluation of the degree of MLP infiltration in the study groups revealed the largest number of cases of severe infiltration (44.5%) in the group with chronic alcoholism, and not a single such case was detected in groups with HIV.

Table 4. Assessment of the degree of infiltration of the mucosal lamina propria by immunocompetent cells, %

Degree of infiltration	Investigated groups		
	HIV infection	Alcohol abuse	Control group
No infiltration	4,7	0	0
Weak infiltration	60,2	12,9	87,5
Moderate infiltration	35,1	42,6	9,1
Severe infiltration	0	44,5	3,4
Total	100	100	100

Results of ICH reaction CD34 realized in cytoplasmic staining with membranous accentuation in all cases of control group with +2 expression (Table 5). But level of expression is reduced in investigated groups. One case has been revealed with negative

results in each investigated group; amount of cases with weak reaction is significant.

Described above factors which could be with presence activities of stromal cells have unclear connection and we have calculated relationship between expression of CD34 and named indicators (table 6).

Table 5. Grading of CD34 for investigated groups.

Grade of expression	Investigated groups		
	HIV infection	Alcohol abuse	Control group
0	1 (4%)	1 (4%)	-
+	5 (20%)	8 (32%)	-
++	19 (76%)	16 (64%)	30 (100%)
Total	25 (100%)	25 (100%)	30 (100%)

Table 6. Relationship between CD34 expression and morphometric indicators, Pearson correlation coefficient (r)

Indicators	Expression of CD34
The thickness of the cervical epithelium	0,64
Relative volumes of condyloma	0,58
Cervical dysplasia severity	-0,81
Degree of infiltration of the mucosal lamina propria by immunocompetent cells	0,68

Discussion. On the basis of the study, it can be assumed that there is a combined effect of factors contributing to the development of pathological processes both in the epithelium and in the mucous membrane of the cervix [5,13]. The effects caused by the influence of confirmed HIV infection are an equally important link in the pathogenic chain of changes [14]. It can be assumed that the main link leading to the development of pathological processes in the cervix in this case is severe immunosuppression, which leads to an increased risk of HPV infection, the development of various types of warts, and the development of dysplastic changes in the cervix [7, 15]. These changes, in turn, are the substrate for thickening of the mucosa, which was the most pronounced in HIV-infected women in our research. It should also be noted that the thickness index was significantly ($p < 0.05$) lower in women who had clinical and history data only on the presence of chronic alcoholism, compared with the group of HIV-infected women. Therefore, it can be assumed that the presence of HIV infection is more significant for the occurrence of a cascade of pathological reactions leading to changes in the cervical mucosa [15,9]. This assumption could be confirmed by the data obtained in the study of the prevalence of various types of genital warts and data on the severity of dysplasia.

So, in the comparison group, the relative volumes of condylomas were minimal and equally distributed over all three types (both pointed, flat and inverted). Genital warts prevailed in the group of HIV-infected patients, pointed and flat warts in women with alcoholism. Genital warts are known to greatly increase the risk of developing oncological processes in the cervix [16,5]. Thus, history data on alcohol abuse, information about the HIV status of a woman and data from a physical examination by a gynecologist can serve as

a basis for suspected presence of contributing factors for the development of malignant neoplasms, which should cause even greater oncological alertness when working with this cohort of individuals [6].

The degree of infiltration of the mucosal lamina propria by immunocompetent cells was determined in all three groups of patients with uneven results. The most pronounced infiltration was determined in women suffering from chronic alcoholism. At the same time, in the control group, as in the group of HIV-infected women, weak infiltration prevailed against the background of chronic alcoholism. These changes can be explained by severe immunosuppression due to HIV infection. Due to this, inflammatory changes do not develop. Consequently, the patients of these study groups are characterized by more dysplastic rather than inflammatory processes in the cervix.

Given the prevalence of severe and moderate MLP infiltration in patients who abused alcohol, it is them who should be expected to have a pronounced presentation of cervicitis. Thus, the data obtained in the course of this study will help to timely and effectively examine women who have risk factors in terms of the development of pathological processes in the cervix, and therefore, to choose a treatment to correct these processes, and hence to increase the duration and quality of life of HIV-infected patients with clinical and history data characteristic of the presence of concomitant chronic alcohol abuse.

There are varying data and slightly different results regarding the expression of CD34 in various precancerous conditions [17]. General consensus is that CD34 appears to be a new and upcoming marker which can be useful in assessing early diagnosis of invasive cervical cancer [12, 10].

The present study was conducted to evaluate the presence of CD34 expression in lesions of uterine cervix under influence of immunodeficiency process both infectious and non-infectious origin. We evaluate presence activities of stromal cells for detection of connection and relationship between expression of CD34 and the thickness of the cervical epithelium, relative volumes of condyloma, cervical dysplasia severity, degree of infiltration of the mucosal lamina propria by immunocompetent cells.

CD34 immunostaining was studied for its pattern of expression and its correlation with named indicators. It was observed that adjacent normal cervical stroma showed dense network of CD34 fibrocyte in the stroma (2+ score). Most close connection (but negative) is observed for cervical dysplasia severity and CD34 expression.

According to literature data early stromal invasion is characterized by a focal loss of CD34+ fibrocytes. So, our result could be used for detection of early potential of malignant transformation tissual changes in women with immunodeficiency and can be used for sensitive tool in detecting tiny foci of stromal invasion in early cancer.

Conclusion.

HIV infection and alcohol abuse have pronounced pathological effects with cervical changes. The expression of CD34 is present in 96% of women with immunodeficiency mainly with strong reaction. It is statistically likely that it does not depend on such morphological indicators as thickness of the cervical

epithelium, relative volumes of condyloma, degree of infiltration of the mucosal lamina propria by immunocompetent cells. The expression of CD34 has statistically close negative connection with cervical dysplasia severity ($r=-0,81$) and can be used for detection of early potential of tissual transformation in women with immunodeficiency.

Conflict of Interest Statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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THE INFLUENCE OF IMMUNODEFICIENCY ON THE LEVEL OF CD34-POSITIVE CELLS IN THE CERVIX

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Summary. There are many studies on changes in cervix, however, there are no data about property of cervical tissue to keep potential for regeneration under influence of immunodeficiency, which is very important since it allows escaping severe irreversible changes in tissue. CD34 stem cells are one of significant indicator for property of tissual regeneration. So, the purpose of our study was to identify amount of CD34 stem cells in the cervix under influence of immunodeficiency of infectious and non-infectious origin.

Materials and methods. Sectional material of reproductive women was studied. All subjects were divided into 3 groups: women who were diagnosed with HIV infection; women who have identified anamnestic and postmortem signs of alcohol abuse; group of comparison. After routine testing and immunohistochemical (IHC) staining to CD34, morphometric measure was performed. We evaluate presence activities of stromal cells for detection of connection and relationship between expression of CD34 and the thickness of the cervical epithelium, relative volumes of condyloma, cervical dysplasia severity, degree of infiltration of the mucosal lamina propria by immunocompetent cells. The obtained digital data were statistically processed.

Results. Morphological investigation revealed changes of thickness of the cervical stratified squamous nonkeratinized epithelium up to $714.23 \pm 59.21 \times 10^{-6}$ m in group of HIV-infected women. Relative volumes of condylomas were

increased in both investigated groups with presence of pointed, flat and inverted types. Both low- and high-grade squamous intraepithelial lesions were detected more often in investigated groups. Assessment of the degree of infiltration of the mucosal lamina propria by immunocompetent cells was changed unevenly with reducing in HIV-group and increasing in alcohol group. Results of ICH reaction CD34 realized in cytoplasmic staining with membranous accentuation in all cases of control group with strong, but reduced level in investigated groups. Most close connection was observed for cervical dysplasia severity and CD34 expression.

Conclusions. HIV infection and alcohol abuse have pronounced pathological effects with cervical changes. The expression of CD34 is present in 96% of women with immunodeficiency mainly with strong reaction. It is statistically likely that it does not depend on such morphological indicators as thickness of the cervical epithelium, relative volumes of condyloma, degree of infiltration of the mucosal lamina propria by immunocompetent cells. The expression of CD34 has statistically close negative connection with cervical dysplasia severity ($r=-0,81$) and can be used for detection of early potential of tissual transformation in women with immunodeficiency.

Keywords. cervix, immunodeficiency, CD34, morphometry

Резюме. Существующие работы об изменениях в шейке матки, не описывают ее свойства сохранять способность к регенерации под влиянием иммунодефицита, что очень важно, поскольку позволяет избежать тяжелых необратимых изменений в ткани. Стволовые клетки CD34 являются одним из важных индикаторов регенеративных свойств тканей. **Целью** нашего исследования было выявление количества стволовых клеток CD34 в шейке матки под влиянием иммунодефицита инфекционного и неинфекционного генеза.

Материалы и методы. Изучен секционный материал женщин репродуктивного возраста. Все исследуемые были разделены на 3 группы: женщины с диагнозом ВИЧ-инфекция; женщины, у которых выявлены анамнестические и патологоанатомические признаки злоупотребления алкоголем; группа сравнения. После рутинной проводки и иммуногистохимического (ИГХ) окрашивания на CD34 проведены морфометрические измерения. Оценивали

наличие активности стромальных клеток для выявления связи и взаимосвязи между экспрессией CD34 и толщиной эпителия шейки матки, частотой кондилом, выраженностью дисплазии шейки матки, степенью инфильтрации собственной пластинки слизистой оболочки иммунокомпетентными клетками. Полученные цифровые данные статистически обрабатывали.

Результаты. Морфологическое исследование выявило изменение толщины многослойного плоского неороговевающего эпителия шейки матки до $714,23 \pm 59,21 \times 10^{-6}$ м в группе ВИЧ-инфицированных женщин. Кондиломы чаще встречались в обеих исследуемых группах с наличием остроконечных, плоских и инвертированных типов. В исследуемых группах чаще выявлялись плоскоклеточные интраэпителиальные поражения как низкой, так и высокой степени. Степень инфильтрации собственной пластинки слизистой оболочки иммунокомпетентными клетками менялась неравномерно, снижаясь в группе ВИЧ-инфицированных и увеличиваясь в группе алкоголя. Результаты реакции ИГХ CD34 с цитоплазматическим окрашиванием и мембранной акцентуацией во всех случаях контрольной группы с сильным были выявлены, но уровень экспрессии в исследуемых группах был снижен. Наиболее тесная связь наблюдалась между степенью тяжести дисплазии шейки матки и экспрессией CD34.

Выводы. ВИЧ-инфекция и злоупотребление алкоголем характеризуются выраженными патологическими изменениями шейки матки. Экспрессия CD34 присутствует у 96% женщин с иммунодефицитом преимущественно с выраженной реакцией. Статистически достоверно, что она не зависит от таких морфологических показателей, как толщина эпителия шейки матки, относительные объемы кондилом, степень инфильтрации собственной пластинки слизистой оболочки иммунокомпетентными клетками. Экспрессия CD34 имеет статистически тесную отрицательную связь с тяжестью дисплазии шейки матки ($r=-0,81$), что может быть использована для выявления раннего потенциала тканевой трансформации у женщин с иммунодефицитом.

Ключевые слова: шейка матки, иммунодефицит, CD34, морфометрия.