

## ORIGINAL PAPERS / PRACE ORYGINALNE

# Cytological transformation of the cervix in immunodeficiency aggravated by alcoholism

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Comorbid pathology, high mortality and disability remain an urgent problem for people with the development of an immunodeficiency state, including women of reproductive age. Prognostic triggers could help to improve management of preneoplastic injury of cervical epithelium.

**The aim** of this study was to identify the cytological features of the cervical epithelium in women with immunodeficiency status and alcoholism, depending on the level of CD4-lymphocytes.

**Material and methods.** The study involved cytological analysis of women, who were divided into 3 groups (group consisted of patients with confirmed HIV infection and chronic alcoholism; group consisted of women with chronic alcoholism; comparison group). Determination of CD4 lymphocytes was carried out with gradation of indicators of 500-350-100 cells per microliter. The relationship between the number of lymphocytes in a smear and changes in epithelial cells was carried out. **Results.** Microscopic examination revealed koilocytosis, binuclear and multinuclear cells, keratinocytes, parakeratosis, cytoplasmic amphiphilia in all women of the two studied groups. In addition, epithelial cells were characterized by large size, displacement of the nucleus to the periphery, uneven staining of the cytoplasm, karyopyknosis, karyorrhexis, karyolysis, and dyskeratosis. The inflammatory type of smear was detected in all women of these groups with the presence of neutrophils, eosinophils, lymphocytes, plasmocytes, histiocytes, macrophages and microflora. At the same time, in the comparison group, the inflammatory type of smear was detected only in 36.6%. When studying the relationship between the level of CD4 cells and the degree of damage to the cervical epithelium in the study groups, a strong correlation was established ( $r=0.824$  and  $r=0.801$ ).

**Conclusions.** With the development of immunodeficiency status, the number of CD4 cells below 100 per  $\text{mm}^3$  should be considered as an unfavorable prognostic factor for the development of neoplastic transformation of the cervix.

**Key words:** cervix, immunodeficiency, alcohol, cytology, CD4

Pol Med J, 2022; L (299); 273–276

## Transformacja cytologiczna szyjki macicy w niedoborach immunologicznych obciążonych przez alkoholizm

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Patologie towarzyszące, wysoka śmiertelność i znaczny spadek jakości życia stanowią duży problem dla osób z upośledzeniem odporności, w tym dla kobiet w wieku rozrodczym. Kryteria prognostyczne mogą okazać się pomocne w terapii zmian przedrakowych nabłonka szyjki macicy.

**Celem badania** było wyszczególnienie odrębności cytologicznych nabłonka szyjki macicy u kobiet z niedoborem odporności i zespołem uzależnienia od alkoholu w korelacji do poziomu limfocytów CD4+.

**Materiał i metody.** Praca badawcza polegała na ocenie wskaźników cytologicznych kobiet, podzielonych na 3 grupy: (grupa chorych z potwierdzonym AIDS i przewlekłym ZZA, grupa kobiet z przewlekłym ZZA, grupa kontrolna). Pomiar ilości limfocytów CD4+ prowadzono według progów ustalonych na poziomie 100-350-500 w  $\text{mm}^3$ . Przeprowadzono analizę korelacji pomiędzy liczbą limfocytów CD4+ w wymazie a zmianami w komórkach nabłonkowych.

**Wyniki.** W badaniu mikroskopowym u kobiet z obu grup badawczych stwierdzono koilocytozę, komórki dwu- i wielojądrzaste, keratynocyty, parakeratozę, amfifilowość cytoplazmy. Jednocześnie komórki nabłonka charakteryzowały się dużym rozmiarem, przesunięciem jądra komórkowego na obwód, nierównomiernym barwieniem się cytoplazmy, kariopyknozą, karioreksją, kariolizą i dyskeratozę. U wszystkich kobiet z grup badawczych w wymazach stwierdzono zmiany zapalne z obecnością neutrofilii, eozynofili, limfocytów, plazmocytozy, histiocytozy, makrofagów i mikroflory. Jednocześnie w grupie kontrolnej zmiany zapalne w cytologii stwierdzono tylko u 36,6% kobiet. W analizie korelacji pomiędzy liczbą komórek limfocytów CD4+ a stopniem zajęcia nabłonka szyjki macicy, w grupach badawczych wykazano silną dodatnią korelację ( $r=0,824$  i  $r=0,801$ ).

**Wnioski.** W stanach niedoboru odporności ilość limfocytów CD4+ poniżej 100 komórek w  $\text{mm}^3$  można traktować jako negatywny czynnik rokowniczy nowotworowej metaplastyki płaskonabłonkowej szyjki macicy.

**Słowa kluczowe:** szyjka macicy, niedobór immunologiczny, alkohol, cytologia, CD4+

Pol Merkur Lekarski, 2022; L (299); 273–276

Condition of immune system depends of various factor [1], which could be realized in combination of pathological processes. Comorbid pathology, high mortality and disability remain an urgent problem for people with the development of an immunodeficiency state, including women of reproductive age [5,24]. In accordance with previous studies, an increased risk [19,25] of the development of cervical intraepithelial neoplasia (CIN) from the functional state of the T-cell immunity.

Often the conditions that characterize alcohol abuse are not regarded as serious abnormal conditions, they are simply not given much importance in the modern world. And even episodes of acute alcohol intoxication, which are associated with formidable changes on the part of many organs and systems, up to the development of coma, do not cause concern. Therefore, chronic alcohol intoxication causes less fear in patients. The lack of a critical attitude towards this disease can

also be associated with the effects of ethanol on the mental state of a person, the development of personality degradation. There are difficulties in organization of screening for such women with point of view of early pathological process detection [20].

At the same time, a significant number of deaths from malignant transformation of the cervical epithelium can be prevented using cytological screening [2,29]. One of the methods for detecting early pathology of the cervix is a regular examination by a gynecologist taking sexual and gynecological history and a Pap test [9,12]. Some scientists believe that immunodeficiency women should undergo cervical screening twice during the first year after being diagnosed with immunodeficiency status, and then regularly, once a year, if the results of previous studies were normal. Women with a high amount of human papillomavirus (HPV) are required to undergo cervical cytology every six months. At the same time, one should understand the difficulties of organizing such screening in women leading an asocial lifestyle [16], which makes it necessary to develop objective methods for assessing the health status of such women.

In connection with the above, the purpose of this study was to identify the cytological features of the cervical epithelium in women with immunodeficiency status and alcoholism, depending on the level of CD4-lymphocytes.

## MATERIALS AND METHODS

The study involved cytological analysis of specimens of 90 women of reproductive age from 20 to 40 years, who were divided into 3 groups of 30 women (the groups were recruited on the principle of randomness). The first study group consisted of patients with confirmed HIV infection. An additional factor affecting the immune status in this group was the presence of chronic alcoholism. The second study group consisted of women with chronic alcoholism. The comparison group consisted of uninfected women who did not have any data on immunodeficiency in their history. HIV infection was verified using a serum enzyme-linked immunosorbent assay (ELISA) with confirmation by Western blotting. When determining less number of CD4 lymphocytes, gradation was carried out with indicators of 500-350-100 cells per microliter. The presence of less than 100 cells was considered "low". The relationship between the number of lymphocytes in a smear and changes in epithelial cells was carried out.

To obtain cellular material, swabs were taken from the vaginal surface of the cervix and cervical canal with a Cervico-

system), which was sent to the laboratory, where thin-layer cytological samples were prepared using special equipment. As a result of a series of procedures (separation of the cell material from the brush, determination of the density of the cell suspension, further dilution in accordance with the density group, centrifugation, and automatic application of the cell suspension on glass), samples were obtained in which the cells were arranged in a uniform monolayer. Evaluation of dysplastic processes in the epithelium of the cervix was carried out according to the Bethesda cytological classification.

Statistical processing 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 in the format  $M \pm \sigma$ , where  $M$  is the arithmetic mean,  $\sigma$  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, 17 October 2011).

## RESULTS

Microscopic examination revealed koilocytosis, binuclear and multinuclear cells, keratinocytes, parakeratosis, cytoplasmic amphiphilia in all women of the two studied groups, which is usually considered as signs of infection of the cervix with highly oncogenic strains of the human papillomavirus. In addition, epithelial cells were characterized by large size, displacement of the nucleus to the periphery, uneven staining of the cytoplasm, karyopyknosis, karyorrhexis, karyolysis, and dyskeratosis. The inflammatory type of smear was detected in all women of these groups with the presence of neutrophils, eosinophils, lymphocytes, plasmocytes, histiocytes, macrophages and microflora. At the same time, in the comparison group, the inflammatory type of smear was detected only in 11 women (36.6%). The distribution of women with identified lesions of the cervical epithelium and the level of CD4 cells are presented in table 1. When studying the relationship between the level of CD4 cells and the degree of damage to the cervical epithelium in the study groups, a strong correlation was established ( $r=0.824$  and  $r=0.801$ ).

**Table 1.** Distribution of women depending on the identified lesions of the cervical epithelium and the level of CD4 cells  
**Tabela 1.** Rozmieszczenie kobiet w zależności od zidentyfikowanych zmian nabłonka szyjki macicy i poziomu komórek CD4<sup>+</sup>

Sign	HIV-infected with chronic alcoholism, N=30	Group with chronic alcoholism, N=30	Comparison group, N=30
Low-grade cervical intraepithelial lesions (L-CIL)	19 (63.3)*	20 (66.7)*	10 (33.3)
High-grade cervical intraepithelial lesions (H-CIL)	11 (36.7)*	10 (33.3)*	1 (3.3)
The number of CD4 in 100 µl more than 500 cells	–	–	30 (100)
CD4 count per 100 µl from 350 to 500 cells	2 (6.6)	4 (13.3)	–
CD4 count per 100 µl from 100 to 350 cells	20 (66.7)	21 (70.0)	–
CD4 count per 100 µl is less than 100 cells	8 (26.6)	5 (16.7)	–

Note: \* the presence of a significant difference relative to the comparison group ( $p < 0.05$ )

Brush. After sampling, the material was applied in a thin layer on glass, dried in air, fixed in Nikiforov's mixture for 10-20 minutes, then stained according to Papanicolaou. The study was carried out on an Olympus BX41 microscope with further morphometric study using the Olympus DP-soft 3.12 software (Japan). Assessment of the state of the epithelium of the cervix was carried out according to the cytological classification according to Papanicolaou [7, 22].

Cellular material for liquid cytology was transferred with a cytobrush into a liquid accumulation medium (CITOSCREEN

## DISCUSSION

The study of disorders of the male and female reproductive system is multifaceted, due to the presence of organ-specific changes [11,26], which vary depending on the etiological factor [27,31], environment [8,14], pathogenesis of the disease [16,33], treatment [21], lifestyle [28]. The female reproductive system, as an integral part of the body, is dependent on the processes occurring in it [6], including the development of an immunodeficiency status [4,10].

Women are a particularly vulnerable group in terms of alcohol abuse. It should be borne in mind that the development of chronic alcoholism in women requires much shorter periods of time than in men, which is due to lower body weight, hormonal factors, and social factors. There are a huge number of reasons that lead to the development of abnormal changes in organs and tissues.

Conventionally, all these reasons can be divided into three groups: the first group includes the reasons associated with the direct action of ethanol, which is known to be a poison that affects all organs and systems. To date, the mechanisms of its influence on the cardiovascular and nervous system have been well studied. However, its impact on the reproductive system has been investigated to a lesser extent.

One of the factors contributing to ovulation is considered to be precisely the production of luteinizing hormone. Thus, hypoproduction of LH can lead to disruption of the menstrual cycle [30], manifested by the development of amenorrhea and dysmenorrhea [3,15]. Therefore, when treating such a group of women, it is necessary to choose a more careful approach to history taking and assume the presence of hormonal imbalance at all levels of regulation (both central – pituitary gland, hypothalamus, and peripheral – ovaries) [17].

Also, changes that occur in the endometrium can be enhanced by the direct toxic effects of ethanol. Ethanol, which is in the composition of all alcoholic beverages, is known to be a poison, which, although it belongs to the 4<sup>th</sup> class of toxic substances (low-risk for humans), in prolonged and significant consumption, can cause a number of abnormal changes. One of its effects is the ability to cause hemolysis up to the development of hemolytic anemia, which in turn can also affect the state of the endometrium, disrupt tissue trophism and stimulate the onset of sclerotic processes in it [18].

The results of our study are combined with data on the choice of prognostic factors for the progression of cervical lesions based on determining the number of CD4+ T-lymphocytes and the age of the patient [13]. However, there is evidence that untreated cervical neoplasia is more likely to progress to invasive cancer in HIV-infected women than in healthy women. Information about the effect of chronic alcoholism on the tumor transformation of cervical cancer is much less known, however, in our previously published results, there are data on a higher proliferative activity of the cervical epithelium and transformation of the apoptotic process in such women.

The chances of relapse after treatment are quite high in the presence of dysfunction of the immune system. Virtually all studies draw attention to the higher incidence of CIN in HIV-infected women compared to uninfected women. According to some reports, CIN is present in more than a third of HIV-infected women, even in the absence of abnormalities in pap smears [23].

A change in cellular blood counts can be one of the triggers not only for an unfavorable course of an immunodeficiency state, but also a marker of the risk of developing comorbidities. Women with a low content of CD4-lymphocytes (less than 200 cells/mm<sup>3</sup>) have the highest prevalence of HPV infection, and more often these are HPV types of high oncogenic risk [2]. This may explain the higher incidence of cervical cancer in immunocompromised patients.

In particular, according to the literature data, women with HIV infection and cervical cancer are not cured of cancer at the same time as successfully as HIV-negative patients. At the same time, it is the indicator of helper cells that corrects with the success of treatment. Women with CD4 counts over 500 tend to recover faster [13].

Moreover, a complex of changes characteristic of a complex transformation throughout the body in the case of the development of immunodeficiency triggers a complex cascade of cellular interactions, one of the stages of which is the morphological restructuring of the cervical epithelium. In general, it can be stated that the acceleration of neoplastic processes of the cervix in immunodeficiency with a decrease in CD4 cells below 100 per  $\mu$ l, which is manifested by an inflammatory type

of smear, koilocytosis, the presence of two- and multinuclear cells, keratinocytes, parakeratosis, cytoplasmic amphophilia, and, key, a change in cell architecture cervical epithelium.

## CONCLUSIONS

With the development of immunodeficiency status, the number of CD4 cells below 100 per mm<sup>3</sup> should be considered as an unfavorable prognostic factor for the development of neoplastic transformation of the cervix.

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**Conflict of interest:** 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.

**Acknowledgements:** Authors would like give personal thanks to Ukrainian Armed Forces for providing security to their families and made finalizing this publication possible; to all Ukrainian people who are united in face of aggression; to all countries and people who support Ukraine in that days. Especial thanks to Poland which takes more than 3 million Ukrainian refugees, women and children mainly.

Received: 22.06.2022  
Revised: 19.07.2022  
Accepted: 19.08.2022

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