DOI 10.26724/2079-8334-2021-2-76-53-57
UCD 616-071+616-006.03+618.14-006+578.27

E.O. Kindrativ, N.I. Henyk
Ivano-Frankivsk National Medical University, Ivano-Frankivsk

PATHOMORPHOLOGY OF CERVICAL INTRAEPITHELIAL NEOPLASIA IN WOMEN WITH INFERTILITY

e-mail: Kindrativ2016@gmail.com

The article presents the pathomorphological features of cervical intraepithelial neoplasia in women with infertility. Cervical biopsy specimens of 250 women were studied. All cases were divided into two groups, differing in papillomavirus infection presence. The patients with tubal, hormonal and combined forms of infertility represent the risk group for the development and progression of papillomavirus infection associated cervical intraepithelial neoplasia. The reduction in the thickness of non-keratinized stratified squamous epithelium to bulk density index of capillaries of the cervix mucous membrane was observed upon increase of cervical intraepithelial neoplasia severity. The regression equation of the dependence of these parameters was defined. This morphological feature may indicate an early stage of tumour development and can be used as an additional criterion for differential diagnosis of cervical intraepithelial neoplasia severity.

Key words: pathomorphology, cervical intraepithelial neoplasia, infertility.

Е.О. Кіндратів, Н.І. Геник

ПАТОМОРФОЛОГІЯ ДИСПЛАЗІЇ ШИЙКИ МАТКИ У ЖІНОК, ХВОРИХ НА БЕЗПЛІДДЯ

У статті викладені патоморфологічні особливості дисплазії шийки матки у жінок хворих на безпліддя. Досліджували біоптати шийки матки 250 жінок з дисплазією шийки матки, хворих на безпліддя. Всі випадки розділені на дві групи, які відрізняються за наявності папіломавірусної інфекції. Особливу групу ризику, щодо розвитку та прогресії дисплазії шийки матки, що асоційована з папіломавірусною інфекцією складають пацієнтки з трубним, гормональним та поєднаною формами безпліддя. Із зростанням ступеня важкості дисплазії шийки матки спостерігається порушення стромально-епітеліальних взаємовідносин, що проявляється зниженням товщини багатошарового плоского незроговілого епітелію до об'ємної щільності капілярів слизової оболонки шийки матки. Відмічається високий зворотній кореляційний зв'язок між співвідношенням цих показників. Дана морфологічна ознака може свідчити про ранній етап розвитку пухлинного процесу і може бути використана в якості додаткового критерію диференціальної діагностики ступеня важкості дисплазії шийки матки.

Ключові слова: патоморфологія, дисплазія шийки матки, безпліддя.

The work is a fragment of the research project "Improving the efficiency of morphological diagnosis of diseases in adults and children in the presence of comorbid pathology", state registration No. 0121U110770.

The real prevention from cervical cancer (CC) is the timely detection and treatment of precancerous diseases [4]. There is a steady increase in the incidence of precancerous lesions and cervical cancer. There is an increase in the cervix precancerous conditions in women under 40 years, especially in the age group from 20 to 29 years, which significantly affects the population indicators [2]. Despite current advances in the diagnosis and treatment of cervical diseases, cervical intraepithelial neoplasia (CIN) remains an important issue in obstetrics and gynecology. The problem of cervical cancer in women with infertility is of particular relevance. In Ukraine, there is a tendency of increasing the number of cases of female infertility [6].

Among the various etiological factors of the process of neoplastic transformation of the uterine cervix (UC), there is a viral infection, in particular, the human papillomavirus (HPV), which has the greatest oncogenic potential [10, 13]. In 1973, Richart RM suggested the concept that all precancerous changes in the cervical epithelium constitute a single pathological process. Richart called it "cervical intraepithelial neoplasia", which progresses to invasive cancer. In recent decades, the pathogenesis of CC was explained by a new concept, according to which, mild CC may be reversible, as it is an inflammatory process with the development of pathological regeneration that corresponds to productive human papillomavirus (HPV) infection. Morphological manifestations of moderate and severe CIN are discussed in the context of neoplasia [8, 11, 12].

HPV infection can also contribute to reduced fertility, the development of infertility, and the emergence of irreversible disorders in the reproductive system, affecting the development of pathology of pregnancy and leading to high perinatal losses [14].

The purpose of the work was to establish the morphological features of cervical intraepithelial neoplasia associated with papillomavirus infection and without infection in women with infertility.

Material and methods. The material of the study consists of biopsy specimens of UC of 250 infertile women with CC, obtained during diagnostic biopsies in the "Carpathian Center for Human Reproduction".

Table 1 presents the distribution of participants of study groups.

Table 1.

Distribution of patients

Degree of CIN severity	Study group, (n=250)	
	Main, (n=157)	Control, (n=93)
Mild (low-grade)	62	48
Moderate	53	33
Severe (high-grade)	42	12

The mean age of women with CIN was 28.32±1.3 years, in the main group – 27.57±1.3, in the control – 29.08±1.3.

The Ethics Committee of Ivano-Frankivsk National Medical University has established that the research performed did not contradict the basic bioethical norms (protocol No. 99/18 dated 07.02.2018).

The methods of detecting HPV infection included real-time PCR with hybridization-fluorescence detection using a set of reagents for the revealing of HPV DNA of 12 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) in scraping from the cervical canal, transformation zone and pathological areas of UC. The result was calculated automatically by the logarithms of the viral genome equivalents (Ig GE) normalized to 10^5 human genomes, distinguishing three types of viral load: <3 Ig per 10^5 cells, 3–5 Ig per 10^5 cells, and >5 Ig GE per 10^5 [9].

Pieces of the UC underwent conventional proceeding, followed by staining with hematoxylin and eosin. Examination of the samples was performed according to the algorithm proposed by Khmelnytsky O.K. (2004).

We used the morphological examination to assess the thickness of the non-keratinized stratified squamous epithelium (NKSSE), the degree of proliferation, differentiation, and polymorphism of epithelial cells, bulk density index of capillaries (DIC) of the cervical mucosa (units in the field of view), and the ratio of NKSSE thickness to DIC. Epithelial cells with mitotic figures (normal and atypical) were counted in 10 fields of view. We evaluated the histospecific signs of HPV, in particular: acanthosis, basal-cell hyperactivity, dys-, hyper-, parakeratosis, binucleated cells, koilocytes, and pathological mitoses. The number of koilocytes was counted in 10 fields of view according to the following scale: <100 was assessed as (+), 100–200 – (++), >200 – (+++) [3].

We studied lymphocytes, lymphoblasts, plasma cells, plasmablasts, and macrophages on azure II - eosin-stained specimens. We evaluated them quantitatively using an eyepiece and object micrometer by the method of point counting at a total magnification of x200 using a 20-point eyepiece-measuring grid (Avtandilov GG, 1990). To determine the connective tissue elements of the cervix, we stained sections with picrofuxin according to Van Gieson, Hart, Masson, and the method of selective staining of fibrin – Zerbino-Lukasevich method [1].

We used the measuring instrument "Mira" (test control) with image analysis based on the software UTHSCA Image Tool for Windows to calibrate metric parameters of cervical tissue.

We subjected the obtained results to statistical processing by methods of variation statistics (arithmetic mean, standard error, standard deviation, confidence interval). Furthermore, we performed the statistical testing of hypotheses to determine the differences between nonparametric features using the χ^2 (chi-square)-criterion and the z-criterion. Correlation analysis was performed basing on the determination of the parametric correlation coefficient. Probability was assessed by Student's T-test. Results with $p<0.05$ were considered statistically significant. We constructed the mathematical model, conducted regression analysis and determined the multiple regression coefficients using the program SPSS. We processed obtained data using Microsoft Access software, Microsoft Excel 2010 (license № 01631-551-3027986-27852).

Results of the study and their discussion. The study of variants of differentiation and mitotic activity of CIN in a standard histological examination did not reveal any features. In both main and control groups of the study, mitotic activity corresponds to the severity of CIN. We rarely detected mitoses under conditions of HPV infection in mild CIN, only in the lower third of the epithelium (1.8±0.4 units in the field of view). In moderate CIN mitoses are detected in both the lower third of the NKSSE and the intermediate layer (2.7±1.1 units in the field of view), which is 1.5 times the indicator of mild CIN ($p<0.05$). We observed mitoses in the entire thickness of the ectocervix: 5.4±2.1 units in the field of view ($p<0.05$)

in case of severe CIN. Among the mitotic figures, there were pathological mitoses: mild CIN – 0.5±0.01 units in the field of view; moderate CIN – 0.8±0.3 units in the field of view; severe CIN – 1.30±0.2 units in the field of view.

The obtained data on mitotic activity are consistent with the results of other studies [5, 11] and indicates that mitotic cycle disorders, in particular the occurrence of pathological mitoses, have prognostic value in assessing the precancerous process: the higher the activity, the more unfavorable the prognosis.

A breach of stromal-epithelial interaction that is manifested by reduction in the thickness of non-keratinized stratified squamous epithelium (NKSSE) to bulk density index of capillaries (DIC) of the cervix mucous membrane was observed upon increase of CIN severity. There is a high inverse correlation relationship between the ratio of NKSSE to DIC in women with infertility ($r=0.95$, $p<0.05$ – with PVI associated CIN; $r=0.98$, $p<0.05$ – with CIN without PVI). This morphological feature may indicate an early stage of tumour development and can be used as an additional criterion for differential diagnosis of CIN severity. Defined regression equation of the dependence of CIN severity on the ratio NKSSE/DIC (y=[-0.0263* X1+3,9209], where y is CIN severity, X1 – index of NKSSE/DIC, $R^2=0.9185$) allows us to accurately determine the severity of the disease to select an adequate amount of therapeutic measures.

Leukoplakia was detected in 85 women with CIN who suffer from infertility; women with a mild degree of CIN represent the highest percentage of revealed leukoplakia — 53.0 %, of which 31.1 % are observed in tubal infertility. Leukoplakia was found in 32 women (37.7 %) with moderate CIN, of which 37.5 % corresponded to hormonal infertility. Eight patients (9.4 %) with severe CIN have leukoplakia; women with hormonal infertility account for 37.5 % of them. Women with uterine infertility make up the lowest percentage of leukoplakia found in all groups. The results of our study are consistent with the results of Nazarenko E.G. (2007), who found that leukoplakia is the most common (25.8 %) pathology of the cervix in various forms of hormonal infertility. However, the method of χ^2-criterion did not reveal a significant effect of infertility on the presence of leukoplakia depending upon the degree of CIN ($\chi^2=15.50$, $p=0.649$).

Warts (condyloma) were found in 75 patients (30.0 %) with CIN; the development of genital warts depends on the degree of CIN and the type of infertility. Three forms of genital warts verified by histological examination of biopsies of the cervix are morphologically divided into: acuminate (condyloma acuminatum), flat and inverted. Thirty-four patients (45.3 %) have diagnosis of acuminate warts, 29 women (38.7 %) suffer from flat warts, inverted warts were diagnosed in 12 cases (16.0 %). We noted the following types of condyloma in the group of women with a mild degree of CIN, depending on the type of infertility: acuminate – 24 (60.0 %), flat – 9 (22.5 %) and inverted – 7 (17.5 %). Tubal and combined infertility dominates among all forms, regardless of the type of genital warts. Flat condyloma predominated in patients with moderate CIN and infertility; they accounted for 66.7 % (18 women). Acuminate ones took the second place — 25.9 %, inverted condyloma were found only in 2 cases (7.4 %).

Tubal and combined infertility are prevalent in both women with moderate and mild CIN with warts. Forms of genital warts in women with severe CIN were distributed almost equally: acuminate and inverted warts in 3 cases (37.5 %) and flat — 2 cases (25 %). Women mainly represent this group of patients with combined infertility. Condyloma acuminata are observed in 66.7 %, flat – in 50.0 % of cases of combined infertility. Inverted warts in 66.7 % are found in women with tubal infertility. Comparison of histological variants of warts shows an increase in the proportion of flat and inverted variants as CIN progresses. Regarding flat condyloma, the results of our study are partially consistent with the data of Kolomiets L.F. (2002), which indicates that intraepithelial condyloma is combined with CIN in 50 % of cases and with preinvasive CC in 5 %. Using the method of χ^2-criterion in our study, we found a significant effect of histological variants of genital warts on the severity of CIN ($\chi^2=9.48$, $p<0.05$).

Characteristic phenotypic features (koilocytes, bi-, and multinucleated cells) represented obvious signs of HPV infection. Koilocytes are localized mostly in groups, foci, sometimes singly or diffusely, spreading in the thickness of NKSSE. Koilocytosis is observed in cervical biopsies in 86 patients (78.2 %) with mild CIN, with moderate – in 57 patients (66.3 %), with severe form – in 12 cases (22.2 %). Analysis of the intensity of koilocytosis indicates a decrease depending on the severity of CIN. Thus, the highest intensity (+++) of koilocytosis was observed in patients with mild CIN (53.5 %). The lowest intensity of koilocytosis (+) was found in 58.3 % of patients with severe CIN. Thus, we found that the number of koilocytes is inversely proportional to the severity of CIN. This information is consistent with data from other studies [3] and can serve as an indication of the productive HPV infection's transition to the neoplastic process.

The vast majority of cases of koilocytosis were found in women with tubal infertility – 101 cases (62.3 %). Moreover, they include 47 women with mild CIN and 39 women with moderate severity. The combined infertility represents 35 cases (21.6 %) and is in the 2nd place. However, the lowest percentage of koilocytosis – 3.1 % (5 women) is due to uterine infertility in women with mild CIN, while there is an absence of koilocytosis in women with moderate and severe CIN on the background of uterine infertility.

Cervical ectopy (CE) was found in 198 women with CIN. The number of women with mild CIN and CE was 92 (46.5 %), women with moderate – 75 (37.9 %) and in the group of severe course, there were 31 women (15.7 %). Analyzing the type of infertility and the detection of CE, it should be noted that hormonal and tubal infertility made up the largest percentages: 47 women (23.7 %) and 46 women (23.2 %) respectively. The lowest number of CE occurred in women with uterine infertility – 25 (12.6 %).

The intensity of koilocytosis depends on the type of infertility and the degree of CIN. The low intensity of koilocytosis was found in 12 patients (13.9 %) with a mild CIN, with medium-grade CIN – in 28 (32.6 %). Patients with tubal infertility predominated among them and were 33.3 % and 46.4 %, respectively. The highest intensity of koilocytosis was observed in 53.5 % of cases (46 patients), 65.2 % of which were also present in women with tubal infertility.

Cervicitis was present in 211 patients with CIN: low-grade – 96 cases (45.5 %), moderate-grade – 78 women (37.0 %), high-grade – 37 women (17.5 %). Women with tubal infertility and cervicitis accounted for 76 cases (36.0 %). Women with hormonal infertility accounted for 44 cases (20.9 %), women with uterine and peritoneal infertility accounted for 31 (14.7 %) and 28 (13.3 %) cases, respectively. In mild CIN, the largest share (32.3 %) of detected cervicitis belonged to patients with tubal infertility, women with hormonal infertility accounted for 19.8 %, and women represented the smallest share (13.5 %) with uterine infertility.

The volume fraction of inflammatory infiltrate in mild CIN associated with HPV infection is not reliably different from this indicator of the control group ($p>0.05$). Elements of cellular infiltrate are mainly represented by lymphocytes, the volume fraction of which is 6.85±1.9 vol% ($p<0.05$), neutrophilic granulocytes 1.08±0.02 vol%, fibroblasts 0.96±0.1 vol%. For plasma cells, their volume fraction slightly increases but is close to the same in the control group. Cervical samples with mild CIN without HPV infection have a higher (2.64±0.2 vol%, $p<0.05$) volume content of neutrophilic granulocytes. According to the method of χ^2 (chi-square)-criterion, cervicitis on the background of mild CIN has no significant effect on the type of infertility when comparing the studied groups ($\chi^2=9.48$, $p=0.903$).

In moderate-grade CIN, the volume fraction of inflammatory infiltrate associated with HPV infection is 15.9±1.8 vol%. In contrast to the mild degree of CIN, we observed a moderate increase in the number of lymphoblasts. The elements of the cellular infiltrate were mainly represented by lymphocytes ($p<0.05$), macrophages ($p<0.05$), and neutrophilic granulocytes ($p<0.05$). In the group of moderate CIN without HPV infection, there is a probable increase in neutrophilic granulocytes and macrophages compared to the main group. The method of χ^2 - criterion revealed a reliable effect of cervicitis on the type of infertility on the background of moderate CIN when comparing the groups ($\chi^2=9.48$, $p=6.27*10^{-5}$).

In the case of severe CIN, lymphocytes (5.66±1.8 vol%) predominated among the elements of the cellular infiltrate. Lymphocytes were located either uniformly diffusely or formed multiple small clusters with neutrophilic granulocytes, macrophages, and plasma cells. Such clusters were subepithelial and aggregated around the capillaries and foci of CE. Severe CIN was characterized by an increase in the volume fraction of lymphoblasts by 2.8 and 1.5 times ($p<0.05$), respectively, the volume fraction in mild and moderate severity. The χ^2 (chi-square)-test showed that a reliable effect of cervicitis on the type of infertility in comparison between the studied groups ($\chi^2=9.48$, $p=0.616$) was absent under conditions of severe CIN.

Local immune responses, which mostly determine the clinical course and completion of the disease, are crucial in the pathogenesis and morphogenesis of cervical dysplasia [7]. There is a clear relation between the functioning of the immune system, the activity of the local immune response, and the state of vaginal microflora [15]. Despite the activation of anti-infective defense mechanisms, the elimination of HPV does not occur, its persistence continues, which is morphologically verified by damage to the epithelial cells of the cervix. Producing reactive oxygen species in the inflammatory focus neutrophils have a high carcinogenic potential, the implementation of which can lead to an increase in the number of mutations in cells and make a significant contribution to the process of carcinogenesis [5]. Our data convincingly indicate an imbalance in the mechanisms of immune control of cellular homeostasis against

the background of HPV infection, which determines the formation and progression of the neoplastic process in NKSSE.

Conclusion

The results of our study confirm the need for a stepwise study of CIN in women with various forms of infertility, in particular using morphometric methods and methods of variation statistics. A special risk group for the development and progression of CIN associated with HPV infection comprised patients with tubal, hormonal, and combined forms of infertility. The regression equation of the dependence of the CIN severity upon the ratio of NKSSE thickness to the bulk density index of capillaries (DIC) (y=[-0.0263 * X1+3.9209], where y is the severity of CIN, X1–NKSSE/ DIC, R^2=0,9185) permits accurate determination of the disease severity and selection of adequate treatment.

References

1. Bahriy MM, Dibrova VA, Popadynets OH, Hryshchuk MI. Metodyky morfolohichnykh doslidzhen. Bahriy MM, Dibrova VA, redaktory. Vinnytsya: Nova knyha; 2016. 328s. [in Ukrainian]

2. Vorobyova LI, Dunayevska VV, Koshmerynska AM. Patohenetychna terapiya tservikalnoyi intraepitelialnoyi neoplazii II-III stupenia vazhkosti. Reproduktyvnaya endokrynologiya. 2015; 4, 74–77. [in Ukrainian]

3. Ershov VA, Viazovaya AA, Narvskaya OV, Lialyna LV, Lysianskaya AS. Povrezhdeniya ploskogo epiteliya sheyki matki pri papillomavyrusnoy infektsii. Infektsiya i immunitet. 2013; 3(1), 79–82. [in Russian]

4. Kryzhanivska AYe, Kindrativ EO, Mykhailiuk IO. Patomorfoz raku shyiky matky IIb stadii pry neoadiuvantniy systemniy polikhimioterapii. Visnyk morfolohiyi. 2013; 2: 406–410. [in Ukrainian]

5. Nesterova Y V, Kovaleva CB, Chudylova HA, Lomtatydze LV, Evhlevskyi AA. Dvoistvennaia rol neitrofilnykh granulotsytov v realizatsii protivoopukholevoy zashchity. Immunologiya. 2012; 5, 281–287. [in Russian]

6. Yuzko OM, Rudenko NH. Stan ta perspektyvy rozvytku dopomizhnykh reproduktyvnykh tekhnolohiy likuvannia bezpliddia v Ukraini. Reproduktyvna medytsyna. 2012; 2, 46–49. [in Ukrainian]

7. Bhat P, Mattarollo SR, Gosmann C, Frazer IH, Leggatt GR. Regulation of immune responses to HPVinfection and during HPV–directed immunotherapy. Immunol. Rev. 2014; 239(1): 85–98.

8. Bode-Lesniewska B, Caduff R. News in der aktuell gültigen WHO-Klassifikation der HPV-assoziierten Zervix-Läsionen. Praxis (Bern 1994). 2017; 106(6): 305–310. doi: 10.1024/1661-8157/a002623.

9. Heid CA. Real-time quantitative PCR. Genome Res. 1996; 6:986–994. doi: 10.1101/gr.6.10.986.

10. Jahic M, Mulavdic M, Hadzimehmedovic A, Jahic E. Association between aerobic vaginitis, bacterial vaginosis and squamous intraepithelial lesion of low grade. Med. Arh. 2013; 67(2): 94–96. doi: 10.5455/medarh.2013.67.94-96.

11. Meiqun J, Jing H, Dong H, Jiang J, Wang M, et al. HLA–DP is the cervical cancer susceptibility loci among women infected by high-risk human papillomavirus: potential implication for triage of human papillomavirus–positive women. Tumor Biology. 2016; 37(6):8019–802. doi: 10.1007/s13277-015-4673-7

12. Ramakrishnan S, Partricia S, Mathan G. Overview of high–risk HPV's 16 and 18 infected cervical cancer: pathogenesis to prevention. Biomed Pharmacother. 2015; 70:103–110. doi: 10.1016/j.biopha.2014.12.041

13. Reich O, Regauer S, Marth C, Schmidt D, Horn L-C, Dannecker C, et al. Precancerous Lesions of the Cervix, Vulva and Vagina Accord ing to the 2014 WHO Classification of Tumors of the Female Genital Tract. Geburtshilfe Frauenheilkd. 2015; 75(10):1018–1020. doi: 10.1055/s-0035-1558052

14. Serrano B, Sanjosé S, Tous S, Quiros B, Muñoz N, Bosch X, et al. Human papillomavirus genotype attribution for HPVs 6, 11, 16, 18, 31, 33, 45, 52 and 58 in female anogenital lesions. European Journal of Cancer, 2015; 51(13): 1732–1741. doi: 10.1016/j.ejca.2015.06.001

15. Souho T, Benlemlih M, Bennani B. Human papillomavirus infection and fertility alteration: a systematic review. PLoS ONE. 2015; 10(5). doi: 10.1371/journal.pone.0126936.

Стаття надійшла 18.04.2020 р.