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Immunohistological chemichal research of the apoptosis and endometrium APUD-system state interreaction in normal and pathological conditions

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We studied the levels of pro- and anti-apoptotic factors (p53, Bcl-2), the content of the EC cells of APUD system in 228 women of reproductive age with endometrial hyperplasia and endometrial cancer. The development of endometrial hyperplasia without atypia was characterized by low activation of local APUD system, and lack of anti-apoptotic protein p53 and increased levels of Bcl-2 in the epithelium of glands and stroma in the endometrium.
In atypical forms of endometrial hyperplasia we observed the 1,5-fold increased expression of Bcl-2 (p<0,05) and the emergence of pro-apoptotic protein p53, and also determined the 5,5-fold increasing the number of APUD cells compared to the physiological level.
In cases of malignant transformation and development of endometrial adenocarcinoma, we noted the 9,8-fold increase of expression of EC cells and 1,3-fold increase of the Bcl-2 level, determined by the high content of protein p53, which indicates a high level of activation APUD system and imbalance between processes of apoptosis and proliferation.
Key words*: endometrial hyperplasia, endometrial cancer, APUD system, EC cells, p53, bcl-2, apoptosis, proliferation.*

According to the domestic and foreign authors data, endometrium hyperplastic processes (hereinafter – *EHP* (V.H.) are among the most urgent ones in the structure of the gynecological pathology with the frequency of 14% to 83% [1, 3]. The topicality of the of this problem is being intensified by the classifications imperfection, endometrium cancer and pre-cancer changes' risks and aetiopathogenetic moments discussions. The broad discussion between pathomorphologists, clinicians and pathophysiologists on the issue of the EHP pathogenesis, which can be observed in the special literature within the recent years, contributes to the scientists' interest in this problem. A lot of scientific papers have been dedicated to the EHP problem; however, the issues of aetiology, pathogenesis, clinical picture, diagnostics and treatment are not settled yet and remain discussable [2, 5].

It is the disease hormonal conception, that is, the oestrogen content increase, that is said to be the leading factor in the EHP development; nevertheless, there is a point of view that the endocrine factor is just the part of the general situation [4, 6].

According to some authors, these are not only the oestrogens which participate in endometrium apoptosis and proliferation processes' regulation, but the biogenic amines and peptide hormones produced by the APUD-system, that is, by the diffuse endocrine system's cells [7]. Apudocites have been detected in many organs and tissues in terms of the normal state, hyperplastic processes and tumors. As for the endometrium, the apudocites are allocated in the glandular epithelium and produce the biologically active substances which regulate the cells proliferation processes [2].

The improvement of the aetiopathogenetic approach to the EHP diagnosticating and treatment with all the pathogenetic stages taken into account is the scientific area in the contemporary gynaecology favouring the improvement of the diagnostics results and the given patients category treatment.

It is the endometrium APUD-system and apoptosis in endometrium in normal and pathological conditions which were ***the subject of our research***.

MATERIAL AND METHODS

228 women with the endometrium pathology detected (the age varies from 18 to 45 years old), the patients of the Centre for general gynecology, gynaecological endocrinology and reproductive medicine of «Feofania» clinical hospital, as well as the patients of the City maternity home № 3, were examined. The patients were examined on the basis of the Order of the Ministry of Health № 676 from 31.12.2004. All the patients were provided with the clinical examination in accordance with the Ministry of Health's regulatory orders. The control group was made of 30 women given a diagnosis of barrenness, who had had the hysteroscopy investigation prior to the extracorporal fertilization, the mentioned above investigation was related to the tube-peritoneal form of barrenness.

Having the diagnosis clarified, all the patients have been divided into groups. Group 1 consisted of 81 (35,5%) women with the simple EH without atypia (hereinafter – *SEHWA* (V.H.); group 2 was composed of women with the complex (adenomatous) endometrium hyperplasia without atypia (hereinafter – *CEHWA* (V.H.), and stood for 33 women (14,7%), group 3 was composed of women with the simple endometrium hyperplasia with atypia (hereinafter – *SEHWIA* (V.H.), and stood for 32 patients (14,0%), group 4 was composed of women with the complex (adenomatous) endometrium hyperplasia with atypia (hereinafter – *CEHWIA* (V.H.), and stood for 33 (14,4%), and finally, group 5 was composed of women with the endometrium cancer (hereinafter – *EC* (V.H.), and stood for 49 (21,5%) cases.

The control groups' patients were diagnosed by means of the ultrasound investigation with the further hysteron-resectoscopy. The obtained results were processed with the histolytic investigation methods involved; the endometrium scrapes were fixed in the 10% solution of the neuter formaldehyde, 4–5 micron slices were coloured with the hematoxylin-eosin; immune histochemical investigation was used for the APUD-cells, p53 protein and Bcl-2 apoptosis inhibitor detection. Dewaxed archival blocks slices, as well as the surgical material fixed in the buffered formaldehyde (pH 6,0) for 20 minutes, were also used.

The serotonin Ab-1 mouse monoclonal antibody, Clon Designation 5HT-H20S, was also applied; it was the Ultra Vision Quanto Detection System (Thermoscientific) that was applied as the visualization system.

The generally accepted methods along with the Student's test were the grounds for the results statistical processing.

RESULTS AND THEIR INTERPRETATION

The data analysis gave the grounds to state that the EC-cells producing serotonin turned out to be the major APUD-cells detected in the endometrium; the EC-cells number defined the proliferation processes' intensity. They were allocated in the following way: in some cases there were the single ones, in some cases there were small clusters of them; the small in size cells with the non-numerous granulas

Table 1

APUD-cells in the endometrium expression level in normal and pathological states

Expression	Proliferation phase	Secretion phase	Group 1 SEHWA	Group 2 CEHWA	Group 3 SEHWIA	Group 4 CEHWIA	Group 5 EC
n	2,2±0,06	2,4±0,07	2,7±0,08	9,6±1,3	13,7±1,1	13,9±1,8	23,9±3,2

allocated largely perinuclear, in some cases – apical (in some cases the entire cell's cytoplasm was occupied).

As for the control group, Apudocites (single hormone producing cells) were detected in the quantity of 2,2±0,06, SEHWA group patients expression intensity equaled the control group level (-2,7±0,08), which is the evidence of the absence of the local APUD-system's stimulation.

While investigating the CEHWA group patients' apudocites, their increase up to 9-10 cells in the visual field was detected. As for the SEHWIA group's women, the apudocites expression intensity stood for 13,7±1,1 cells in the visual field (in case of the CEHWIA group – 13,9±1,8 cells correspondingly) which indicates the local APUD-system stimulation.

The maximum increase of the apudocites was detected among the EC group patients, – 23,9±3,2 cells in the vision field, which is 9,8 times as much as the corresponding index for the control group. Thus, the local APUD-system EC-cells maximum expression indexes were observed in cases of atypical forms of the endometrium hyperplasia and among patients diagnosed with the endometrium adenocarcinoms which indicates the overloading of the misleading proliferation suppression.

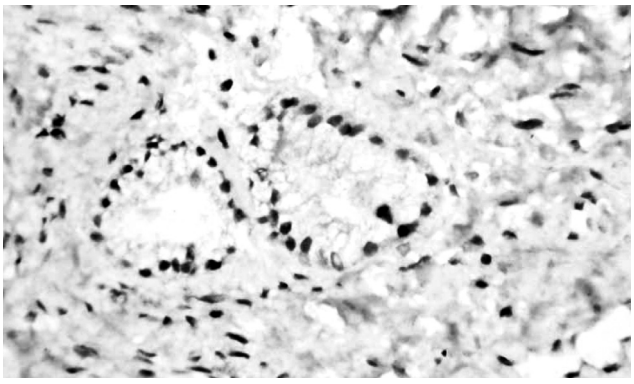
The study of the endometrium apoptosis processes states on the basis of the Bcl-2 apoptosis inhibitor and p53 pro-apoptosis protein in the endometrium cells expression was the next stage of our research.

It was proved that the Bcl-2 protein expression level in the endometrium cells depends on the menstrual cycle phase and the pathological process's character.

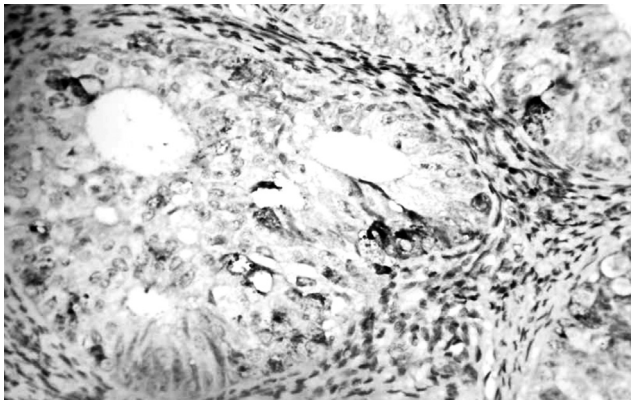
The Bcl-2 protein high content was observed within the proliferation phase of the menstrual cycle in case of the absence of the endometrium pathological process; at the same time, the late secretion phase witnessed the dramatic decrease of this protein level content which leads to the program death of the endometrium unchanged cells by means of the apoptosis. Thus, Bcl-2 protein level in the endometrium glands was 5,6 times as lower as the corresponding index for the proliferation stage; the stroma component exposed the 3 ($p<0,05$) times' decrease of this protein content. It gives all the grounds to state that the Bcl-2 anti apoptosis protein level depends on the menstrual cycle's phase and its content fluctuation is an important factor for the balance of the apoptosis and proliferation in terms of physiology.

In case of the endometrium hyperplastic process's development, there is the balance fluctuation between the apoptosis and proliferation processes, which is characterized by the increase of the Bcl-2 protein level content in endometrium. Thus, the Bcl-2 anti-apoptosis level's index for the SEHWA group equaled the normal endometrium proliferation phase index, and was 5 times as much as the secretion phase's index; the same tendency was observed in the CEHWA group where this increase stood for 5,6 times.

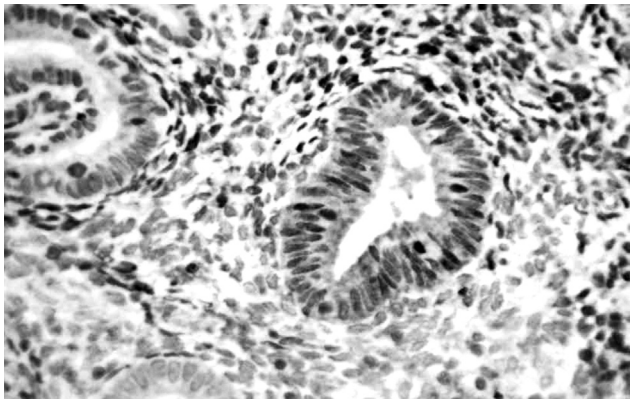
In case of the atypia, SEHWIA and CEHWIA groups showed the significant of the Bcl-2 expression, almost 1,5 times compared to the proliferation phase, and 8 times compared to the secretion phase ($p<0,05$). The Bcl-2 level fluctuation in the stroma component showed the same tendency towards the glands' epithelium, its level was 2 times higher in case of the non-atypical EHP forms and 5 times higher in case of the atypical forms.



Picture 1. Local APUD-system EC-cells in the endomentrium for normal state



Picture 2. Local APUD-system EC-cells in the endomentrium for the endometrium adenocarcinoma



Picture 3. Local APUD-system EC-cells in the endomentrium for the complex endometrium hyperplasia without atypia

Table 2

Bcl-2 expression level in the endometrium in normal and pathological states (points)

	Proliferation phase	Secretion phase	Group 1 SEHWA	Group 2 CEHWA	Group 3 SEHWIA	Group 4 CEHWIA	Group 5 EC
Glands	1,7±0,04*	0,3±0,04*	8	1,6±0,08*	1,7±0,09*	2,5±0,08*	2,6±0,08*
Stroma	0,6±0,03	0,2±0,03	0,3±0,01	0,4±0,08	0,9±0,03	1,1±0,03	1,1±0,07

*reliability of deviation ($p<0,05$).

Table 3

P53 protein expression in the endometrium in normal and pathological states

	Control group	Group 1 SEHWA	Group 2 CEHWA	Group 3 SEHWIA	Group 4 CEHWIA	Group 5 EC
Glands %	-	-	-	6,4±1,1*	8,2±0,7*	38,5±8,3*
Stroma %	-	-	-	5,9±0,7*	7,3±0,3*	32,8±6,4*

*reliability of deviation ($p<0,05$)

Bcl-2 level study among endomentrium cancer patients showed definite specific features, namely, statistically broad indexes amplitude, lowering of atypical expression level in case of the high differentiated carcinomas, and the given protein high indexes in case of the low differentiated ones. In our opinion, this fact is determined by the loss of proliferation control mechanisms by the epithelium influenced by malignant process; that is, by the appearing of the Bcl-2 negative cells with the uncontrolled proliferation and the malignant proliferation process symptoms.

It is the p53 protein which is of great importance for the provision of the balance between proliferation and the programmed cells' death; this protein is the product of TP-53 tumor gene-suppressor and is expressed in all the cells of the organism. In case there are no genetic apparatus disorders, this protein is inactive, but if the DNA is damaged, p53 shows expression. This protein is activated in case of the DNA disorders accumulation, its activation results in the cells cycle termination, DNA replication and apoptosis launch. Thus, the p53 protein function is the deletion of the cells which are considered to be the potentially cancer causing ones out of the pool; that is why we think the study of its influence on EHP development is the important stage of the given pathology pathogenesis's consciousness and the pathological process character estimation.

P53 protein in the endometrium was detected as the brown pale colouring of the cells' nucleus (core). The given protein expression was not detected in the SEHWA, CEHWA and control group which indicated the presence of cells with the atypia features.

The strong tendency towards the p53 protein expression growth was detected in case of the atypical endometrium hyperplasia forms, especially, with the maximum indexes for EC. Thus, the given protein level stood for 6,4% for SEHWA group and 8,2% for the CEHWIA one; in case of the malignant transformation, the protein level showed dramatic increase, up to 38,5% which indicated the accumulation of cells with the damaged DNA, malignant transformation of the proliferation process and the apoptosis activation.

Thus, the obtained results of our research give all the grounds to state that there are the pathogenetic correlation and interrelation between the apoptosis process and the intratissular APUD-system state.

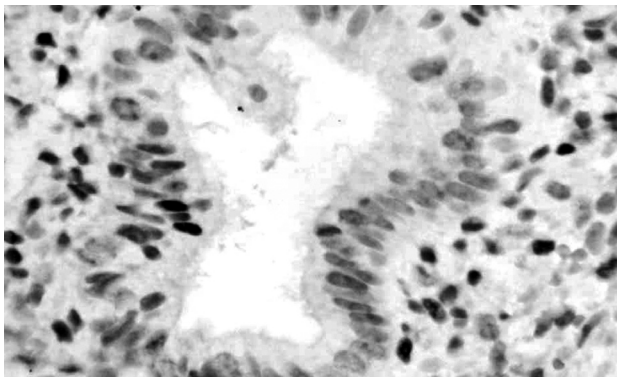
APUD-system cells (EC-cells) along with the pro- and anti-apoptotic proteins sustain the necessary intratissular level of the serotonin content to provide the balance of the proliferation and apoptosis processes in case the endometrium hyperplasia absence.

In case of the hyperplastic process development and the endometrium oncological transformation the APUD-system EC-cells quantity increases, as well as the p53 pro-apoptotic protein, which is of the undirectorial compensatory nature, and aimed at the proliferative process and apoptosis enforcement retardation (Picture 6). on the other hand, the Bcl-2 anti-apoptotic protein level pathological increase proves the lack of the apoptosis processes, the prevalence of proliferation over apoptosis, and, as the result, determines the pathological process's proliferative nature of the .

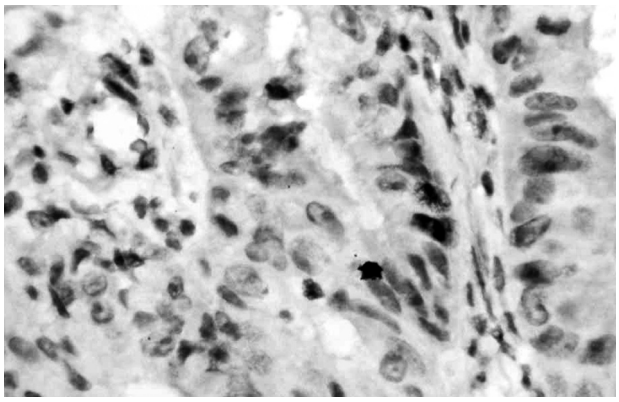
CONCLUSIONS

Thus, as for the reproductive age women, the EC-cells expression in endometrium stands for – 2,4±0,07 in the visual field, which indicates the low physiological (basal) level of the endometrial APUD-system's activation.

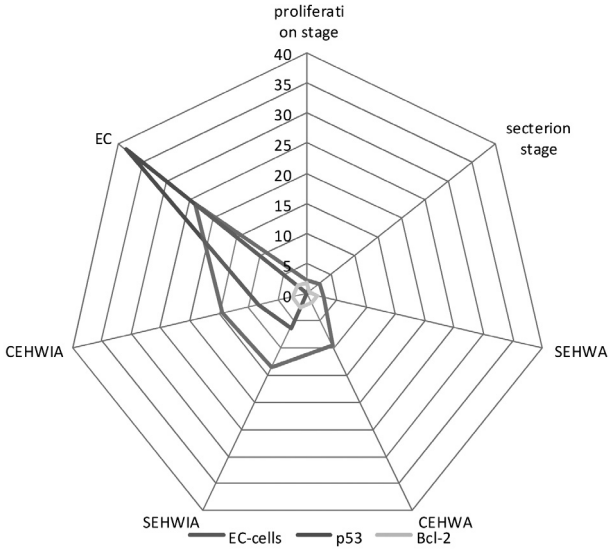
Endometrium hyperplasia without atypia's development is characterized by the low level of the local APUD-system activation, absence of the p53 pro-apoptotic protein and insignificant increase of the Bcl-2 anti-apoptotic protein level in the glands epithelium and endometrium stroma.



Picture 4. P53 expression for the complex endometrium hyperplasia with atypia. Zoom ×40



Picture 5. P53 expression in case of the endometrial adenocarcinoma. Zoom ×40



Picture 6. Comparison of the pro- and anti-apoptotic factors levels with the APUD-system EC-cells expression depending on the pathological process nature

In case of the endometrium hyperplasia atypical forms' development, SEHWIA and CEHWIA, the increase of the Bcl-2 expression (1,5 times increase ($p<0,05$), p53 pro-apoptotic protein presence and the APUD-sells quantity increase (almost up to 5,5 times as much as for the physiological level) are observed. In case of the tumor transformation and the endometrium adenocarcinoma development the EC-cells expression is 9,8 times higher, Bcl-2 protein level is 1,3 times higher, the high content of the p53 protein is detected; all these indicate the APUD-system high level of activation and the apoptosis and proliferation processes' misbalance.

The development of the contemporary endometrium hyperplastic processes pathogenetic model should take into consideration the APUD-system intratissular state and anti- and pro-apoptotic factors as ones of the important constituents of the given pathology development.

The APUD-system EC-cells quantity identification and the apoptosis activity indexes may be used as the integrative diagnostic criterion for the endometrium proliferative process's nature.

Імуногістохімічне дослідження взаємовідносин процесів апоптозу та стану APUD-системи ендометрія в нормі та при патології

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Проведено дослідження вмісту про- та антиапоптичних факторів (p53, Bcl-2) та вмісту ЕС-клітин внутрішньотканинної APUD-системи, у 228 жінок репродуктивного віку з гіперпластичними процесами ендометрія та раком ендометрія. Розвиток гіперплазії ендометрія без атипії характеризується низьким рівнем активації місцевої APUD-системи, відсутністю антиапоптичного протеїну p53 та підвищенням рівня Bcl-2 в епітелії залоз та в стромі ендометрія.

При розвитку атипових форм гіперплазії ендометрія спостерігається підвищення експресії Bcl-2 в 1,5 разу ($p<0,05$) та поява проапоптичного протеїну p53, при цьому визначається підвищення кількості APUD-клітин – в 5,5 разу по відношенню до фізіологічного рівня.

При пухлинній трансформації та розвитку аденокарциноми ендометрія спостерігається збільшення експресія ЕС-клітини в 9,8 разу, збільшення рівня Bcl-2 в 1,3 разу, високий вміст протеїну p53, що свідчить про високий рівень активації APUD-системи та про дисбаланс між процесами апоптозу та проліферації.

Ключові слова: *гіперплазія ендометрія, рак ендометрія, APUD система, ЕС-клітини, p53, bcl-2, апоптоз, проліферація.*

Иммуногистохимическое исследование взаимоотношений процессов апоптоза и состояния APUD-системы эндометрия в норме и при патологии

В.А. Бенюк, О.В. Каленская, В.Н. Гончаренко, А.Н. Строкань, Р.В. Бубнов

Проведено исследование уровня про- и антиапоптических факторов (p53, Bcl-2) и уровень экспрессии ЕС-клеток APUD-системы эндометрия, у 228 женщин репродуктивного возраста с гиперпластическими процессами эндометрия и раком эндометрия.

Установлено, что развитие гиперплазии эндометрия без атипии характеризуется низким уровнем активации местной APUD-системы, отсутствием проапоптического протеина p53 и повышением уровня антипоптического Bcl-2 в эпителии желез и в строме эндометрия.

При развитии атипичных форм гиперплазии эндометрия наблюдается повышение экспрессии Bcl-2 в 1,5 раза ($p<0,05$), появление проапоптического протеина p53, при этом определяется повышение количества APUD-клеток – в 5,5 раза в сравнении с уровнем контрольной группы.

При злокачественной трансформации процесса и развитии аденокарциномы эндометрия наблюдается увеличение экспрессия ЕС-клеток в 9,8 раза, увеличение уровня Bcl-2 в 1,3 раза, определяется высокое содержание протеина p53, что свидетельствует о высоком уровне активации APUD-системы и про выраженый дисбаланс между процессами апоптоза и пролиферации.

Ключевые слова: *гиперплазия эндометрия, рак эндометрия, APUD-система, ЕС-клетки, p53, bcl-2, апоптоз, пролиферация.*

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