CLINICAL AND MORPHOLOGICAL ASPECTS OF THE REPRODUCTIVE SYSTEM OF REPRODUCTIVE AGE WOMEN WITH MENSTRUAL DISORDERS AFTER COVID-19

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Summary

Objective: to study the structural and metabolic features of endometrial structure in reproductive age women with menstrual disorders after COVID – 19 and to determine the correlation between histological status and parameters of endometrial morphometry in this group of patients.

Materials and methods. Bibliographic, information-analytical, clinical-instrumental, laboratory, histomorphological methods were used in the work. Sources of information were data from the scientific literature on the topic of the study, modern gadleins, a review of randomized controlled trials.

Results and discussion. Menstrual disorders such as menometrorrhagia is one of the main reasons for seeking medical help and hospitalization. A significant role of the menometrorrhagia development belongs to the disruption of hormonal homeostasis with changes in the growth and differentiation of cellular elements of the endometrium. Stressors can activate the hypothalamic-pituitary-gonadal axis (HPG axis) and alter the neuromodulatory cascade that controls the regulation of gonadotropin-releasing hormone (GnRH). In addition gonadotropin-releasing hormone (GnRH) may play a role in the autocrine regulator of carcinoma cell line proliferation [13]. According to the WHO more than 106 million patients suffered from COVID – 19 worldwide in 2021. Fear of contracting the virus, forced social exclusion, as well as insecurities about financial stability and the future, have had a significant impact on the mental health of many people in European countries. [1,3,4]. As stress has its own characteristics for the female body we study the structural and metabolic characteristics of hyperplastic processes in reproductive age women with menstrual disorders as menometrorrhagia after COVID – 19 to improve the algorithm of examination and prediction of pathological processes of the endometrium [2,5,6,7].

Conclusions. The described histological picture indicates the presence of asynchrony of the processes of secretory transformation of the endometrium into the luteal phase of the MC. The period of endometrial monitoring in patients of reproductive age with menstrual irregularities by type of menometrorrhagia after COVID-19 should be at least 3-6 months after surgery, as a probable increase in the risk of recurrence of GE over time.

Key words: menometrorrhagia, endometrial hyperplastic processes, menstrual disorders, COVID-19 pandemic, psychological distress.

INTRODUCTION

According to current scientific findings, abnormal or dysfunctional uterine bleeding in 10-25% is manifested against the background of the structural pathology of the endometrium [12]. Hyperplastic endometrial processes are the cause of menometrorrhagia in 40% of women of reproductive age. The very menometrorrhagia is a leading clinical symptom and the main motive for visit to the doctor or hospitalization in case of endometrial
hyperplastic processes [12,11]. Among the reasons for the development of endometrial hyperplasia (EH), there are many important factors that play a role in the disruption of hormonal homeostasis against the background of stress factors. The stress-system is a folding regulatory complex that helps to coordinate homeostasis under normal conditions and plays a key role in activating and coordination of all adaptive changes in the body to stressors [6,7]. Depending on the current data, the system is formed from the central section and two peripheral branches, which carry out the connection of the central section with the whole organism. The central section is located in the brain: in the hypothalamus and other parts of the brainstem. For the female organism, stress may have its own characteristic features, because estrogens have a stimulating effect on the hypothalamic-pituitary-adrenal system, and this reactivity is much higher than in men. Interesting is the discovery of gonadotropin-inhibiting hormone, which is produced under the influence of stress and is the strongest suppressor of fertility. Its analogue, RFRP (RFamide-relatedpeptide), found in mammals and isolated in 2009 in humans, is synthesized in the hypothalamus and reduces the synthesis and release by the pituitary gland of gonadotropins (follicle-stimulating and luteinizing hormones). The hypothalamus has areas associated with the regulation of adaptive, emotional and sexual behavior, determines the correct frequency of functions that are associated with reproduction. One of the most important functions of the hypothalamus is the regulation of pituitary activity. The presence of neural and humoral connections between the hypothalamus and the pituitary gland allowed them to unite into a single hypothalamic-pituitary system. The hypothalamic-pituitary system is of great importance, in particular regulates trophic, growth and reproductive functions of the body, and hypothalamic-metaadeno-pituitary and hypothalamic-post-pituitary systems are most pronounced in stressful situations and thus are directly related to the regulation of protective and adaptive responses. The function of the hypothalamic-pituitary system is controlled by the neurons of the centers of the hypothalamus, also by the brainstem and the higher sections of the CNS (central nervous system), such as the paleocortex. The main result of the activation of the stress system is the increase of release of glucocorticoids and catecholamines—the main stress hormones that help mobilize the function of organs and tissues responsible for adaptation, and increase their energy supply. The data from the last 5 years show that stress affects women’s health with conditions such as infertility, miscarriage, endometrial and mammary gland pathology, early menopause, severe menopause. Stress-induced elevated cortisol levels lead to decreased levels of sex-steroid-binding globulin, which directly leads to lower levels of total testosterone, increased levels of biologically active testosterone, decreased estradiol levels and impaired progesterone synthesis (pregnenolone primarily synthesizes cortisone, not progesterone), which, in general, contributes to the disruption of apoptosis in endometrium and the development of its hyperplasia [11].

According to the WHO and the International Society of Gynecologists-Pathomorphologists, EH is classified on the basis of the presence or absence of cellular atypia, as well as depending on the degree of tissue structure disorders (simple or complex EH). In the last decade, EH treatment, depending on the histological form of the hyperplastic process, is performed either by conservative (progestogen drugs or GnRH agonists) or surgical methods (hysteroscopic subtotal endometrial resection or hysterectomy).

Objective: to study the structural and metabolic features of endometrial structure in reproductive age women with menstrual disorders after suffering from COVID – 19 and to determine the correlation between histological status and parameters of endometrial morphometry in this group of patients.

MATERIALS AND METHODS

The investigation was done during 2020-2021 years in the department of minimally invasive surgery of the State Scientific Institution «Scientific and Practical Center of Preventive and Clinical Medicine» of the Agency of State Affairs. In working with patients followed the ethical principles of the Declaration of the World Medical Association (World Medical Association Declaration of Helsinki, 2000), EEC Directive № 609 (1986), the basic provisions of the «Rules of ethical principles of scientific medical research with human participation», approved by ICHGCP (1996) and orders of the Ministry of Health of Ukraine № 690 dated 23.09.2009, № 944 dated 14.12.2009, № 616 dated 03.08.2012. Each patient signed a voluntary informed consent to the processing of personal data of medical records and to participate in research. The Ethics Committee of the research base of the State Scientific Institution «Scientific and Practical Center of Preventive and Clinical Medicine» of the Agency of State Affairs, received ethical approval to conduct research on the study of menstrual function in women who underwent COVID-19 (protocol № 1 from 31.01.2022). The study included 45 women of reproductive age (30.4±2.3 years).

The inclusion parameters were:
- reproductive age 25-45 years;
- hyperplastic processes of the endometrium and/or menometrorrhagia after suffering from COVID – 19 (within the last year);
- the presence of specific IgG to SARS-CoV-2;
- the desire to preserve and implement the reproductive function;
- the presence of ovaries and a uterus functionally capable of carrying a pregnancy.

Evaluation of specific immunoglobulins IgG and IgM to SARS-CoV-2: detection of IgG and IgM
to SARS-CoV-2 coronavirus nucleocapcid antigen in serum was performed using the method of indirect enzyme-linked immunosorbent assay according to the attached instructions. Enzyme immunoassay test system (HUMAN Gesellschaft fur Biochemica & Diagnostica mbH, Germany) was used and laboratory examination was conducted on the basis of State Scientific Institution «Scientific and Practical Center of Preventive and Clinical Medicine» of the Agency of State Affairs. The diagnosis of EH was established on the basis of pathohistomorphological examination of endometrial biopsy material performed during medical and diagnostic hysteroscopy in women menometrorrhagia after suffering from COVID –19. Surgical treatments were performed using the equipment of the companies «KarlStorz» (Germany) according to the generally accepted recommendations of international and national protocols for medical care and the protocols of the Ministry of Health of Ukraine [протоколи]. Endometrial tissue fragments were fixed in a 10% solution of neutral buffered formalin. A carousel-type STP-120 histoprocessor was used to hold the material after fixing, an EC-350 station was used to cut the paraffin blocks, and a rotary microtome of the HM-340E series (Microm, Hamburg, Germany) was used to cut the paraffin blocks. Histological preparations were stained with hematoxylin–eosin. An Axioskop 40 microscope with an AxioCamMRc5 camera (CarlZeiss) was used. The results of immunohistochemical reactions were evaluated using a semi-quantitative morphometric method. Visually evaluated the intensity of cell staining in points from 0 to 3 (negative, weak, moderate and pronounced reaction) and counted the percentage of positively stained cells at each value of color intensity. 1000 cells in 10 fields of view with the most pronounced immunohistochemical response under microscope magnification 400. Also determined the average area of expression in percent – the ratio of the area with immunohistochemical reaction to the total area of cells / nuclei in the field of view. The assigned parameters reflect the intensity of synthesis and accumulation of hormones and signaling molecules in cells and tissues. For evaluation, was studied the proliferation of endometrium on the basis of histological characteristics, data of morphometry, nuclear DNA, as well as assess the correlation between the histological status and parameters of morphometry and DNA cytometry, and paraffinized the samples of endometrium. The follow-up was carried out with the aid of the MIAMED-DNA Image Analysis analyzer. Was evaluated the cell lining of the endometrial glands, according to which the diagnosis of simple or complex EH, the size and shape of the nuclei, the DNA content. Particular attention was paid to the amount of normal diploid DNA. After measuring 300 nuclei of endometrial cells in the sample were analyzed: a) morphometric parameters – the perimeter of the nucleus, the minimum and maximum transverse dimensions, shape (degree of roughness or sphericity); b) DNA cytometric parameters: average DNA content, DNA index (content of normal diploid DNA), index of deviation of diploid DNA (index of proliferative activity of cell population), number and percentage of nuclei with more than 5-ploidy DNA. To process the data, we used standard statistical methods of multidimensional mathematical analysis – parametric and nonparametric statistics, performed calculations of the arithmetic mean (t) of the sample; standard deviation (s). When comparing data from different clinical groups in order to identify statistical differences in one or another indicator, Student’s t-test calculations were used for the average values of the two groups with the calculation of the probability of error (p); for quantitative traits that do not satisfy the conditions of normal distribution – nonparametric methods of statistical analysis Whitney – Mann.

RESULTS

All patients in the study groups had an ultrasound monitoring of the endometrium at the time of admission and its echo-structural changes in the dynamics of one or two menstrual cycles after surgery.

According to the ultrasound results the thickness of the endometrium was on average 14.8 ± 0.4 mm, which indicated a thickening of the endometrium on the background of the detection of hyperestrogenism characteristic of EH. Analyzing the data of medical and diagnostic hysteroscopy, we can conclude that: EH was diagnosed in 42.3% woman; endometrial polyp (EP) – in 31.0%; signs of chronic endometritis (CE) – in 8.9%, which does not contradict many scientific studies that link the pathogenesis of EP with inflammatory processes of the endometrium. The obtained results are presented in Table 1.

| Endometrial characteristic | n=45(100%) |
|---------------------------|------------|
| Does not correspond to the phase of the menstrual cycle (%) | 37,0(82,3%) |
| PE (%) | 14,0(31,0%) |
| EH (%) | 19,0(42,3%) |
| CE (%) | 4,0(8,9%) |
| EH with follicles endometriosis (%) | 4,0(8,9%) |
| Intrauterine infections (%) | 4,0(8,9%) |

Table 1
Samples in women of reproductive age with menometrorrhagia were observed in the interstitium. Membranes of the endometrium. Diapedetic hemorrhages of immunocomplexes was found on the vascular subepithelial arteries with thickened walls and with the presence of fixed mitochondria with a reduced number of cristae. Clusters of compaction of the cytoplasm, enlightenment of the matrix of perinuclear space, marginal location of heterochromatin, invagination of the nuclear envelope, expansion of signs of perivascular deciduous reaction and with glands location of cells with low proliferative activity. In some – was visualized with areas of fibrosis, with dense and with a wide lumen and a large amount of secretion, in one field of view: in some areas was compact, spongy and vessels in different parts of the endometrium differed figures of mitosis. The structure of the glands of the stroma was characterized by phenomena associated with increased functional activity of cells in the form of «spots», characteristic of p-RNA amplification [8]. A characteristic feature was an increase in secretory epitheliocytes. In most observations, the structure and relative position of the glands varies within a single biopsy, a significant portion of the glands are cystically enlarged. There are tortuous glands, sometimes scalloped, star-shaped, which are more in line with the secretory phase, and straight glands with a narrow lumen. Cystic enlarged glands may be surrounded by a «collar» of densely arranged elongated stromal cells. In the lumen of the glands, often cystic-dilated, watery secretion accumulates. The secretory cells were spherical, uniformly dilated, with a wide lumen and abundant mucus content in them. The structure of the endometrial stroma was characterized by a significant number of neutrophils and lymphocytes. The correlation analysis revealed a strong relationship \( r = 0.8; p < 0.01 \) between plasma cells and lymphocytes, as well as a medium-strength correlation \( r = 0.5, r = 0.5, r = 0.5 \) (\( p < 0.05 \)). Some epitheliocytes contain basal vacuoles in the cytoplasm; there is a secretory epitheliun with signs of apocrine secretion, especially in cystic-enlarged glands lined mainly with proliferating, non-flattened epitheliun with signs of pseudostratification and figures of mitosis. The structure of the glands of the stroma and vessels in different parts of the endometrium differed in one field of view: in some areas was compact, spongy structure of the stroma with dilated, convoluted, spherical and with a wide lumen and a large amount of secretion, in some – was visualized with areas of fibrosis, with dense location of cells with low proliferative activity, without signs of perivascular deciduous reaction and with glands with signs of cell destruction: nucleolus microsegregation, invagination of the nuclear envelope, expansion of perinuclear space, marginal location of heterochromatin, compaction of the cytoplasm, enlightenment of the matrix of mitochondria with a reduced number of cristae. Clusters of arteries with thickened walls and with the presence of fixed immunocomplexes was found on the vascular subepithelial membranes of the endometrium. Diapedetic hemorrhages were observed in the interstitium.

Analyzing the biopsy data of the studied endometrial samples in women of reproductive age with menometrorrhagia after suffering from COVID-19 can be concluded that among 51.1% of clinical observations there is a synchronous hyperplastic transformation of the endometrium (mainly due to glandular component) in 48.9% cases – asynchronous development of endometrial hyperplastic processes (associated with stromal and glandular components).

In the postoperative period (3 months after surgery) a control histological examination of endometrial aspirate biopsy was performed: in two patients with simple EH was determined complex EH. Six months after treatment the expected recovery of the functional state of the endometrium occurred in most cases \( (n=22, 66.7%) \) among all cases of endometrial hyperplastic processes \( (n=33, 100%) \). Recurrence of hyperplastic changes of the endometrium in women 6 months after surgery was calculated by the EH risk factor. Despite a small sample, there was a likely increase in the risk of developing EH over time (from 1.0 3 months after surgery and 1.0 6 months after surgery to 2.07 after 6 months of the control period and to 18.3 after 12 months) months, \( p < 0.01 \), which is a consequence of the progression of the abnormal proliferative state, perhaps because of maintaining the influence of stress factors.

Statistical analysis of variations of all parameters revealed that, except for the form factor and DNA index, the dynamics of all other parameters were statistically significant \( (p<0.001) \). The Spearman correlation coefficient was calculated for all parameters. A clear probable negative correlation was found between time and all DNA parameters \( (DNA \text{ index}: r=-0.49, p<0.001; \text{diploid DNA deviation index}: r=-0.50, p<0.001; \text{number and percentage of nuclei with more than than with 5-ploid DNA}: r=-0.66, p<0.001) \). A weak, but also a negative correlation with the time was revealed \( (r=-0.16, p<0.05) \) only for one morphometric parameter – the minimum transverse size of the nucleus.

A negative correlation was found between the histological picture of the endometrium and the morphometric parameters of the nuclei. With a probability level of \( p<0.05 \) on the average value of the form’s factor, the sample with recurrent hyperplasia after 3 months of postsurgical treatment differed from samples with functional endometrium, and the DNA index, perimeter of the nucleus, form’s factor and minimum transverse nucleus size the sample with 3 through months after surgery differed from cases without hyperplasia.

**CONCLUSIONS**

In the current scientific literature, the results of only a small number of studies of the functional state of the endometrium in reproductive age women with menstrual disorders as menometrorrhagia after suffering COVID-19 have been shown. Therefore, the study of this issue at the modern level, using the latest informative methods, is of undoubted relevance.
The period of observation of the state of the endometrium in patients of reproductive age with menstrual cycle disorders according to the type of menometrorrhagia after suffering from COVID-19 should be at least 3-6 months after surgery, since it has been established that the risk factor of GE recurrence increases over time.

The described histological picture indicates the presence of asynchronization of the processes of secretory transformation of the endometrium into the luteal phase of the MC. Therefore, in general, asynchronous structure of glandular, stromal layers and vessels was noted in different sections of the same field of vision. The structure of the endometrium indicated a deepening of the processes of separation of the structural secretory maturation of the glands and the stroma, due to the uneven development of the glandular and stromal components. When assessing the cellular composition of the stroma in patients with both synchronous and asynchronous retardation of endometrial development, a general tendency was revealed to increase the number of cells of specific resistance — lymphocytes and plasma cells, with a simultaneous decrease in the number of cells of non-specific resistance — segmented nuclear leukocytes, i.e. disconnection or lack of cooperative connections between specific and non-specific links of immunity.

Summarizing the data on endometrial morphometry, it can be stated that there is a break in not only horizontal, but also vertical connections of the links of cellular immunity – specific and non-specific, which indicates a complete discoordination of the cellular link of immunity in the endometrium in women of reproductive age with disorders of the menstrual cycle according to the type of menometrorrhagia after suffering from COVID-19.

PARTICIPATION
Both authors participated in drafting the article.

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Резюме

КЛІНІКО-МОРФОЛОГІЧНІ АСПЕКТИ СТАНУ РЕПРОДУКТИВНОЇ СИСТЕМИ ЖІНОК РЕПРОДУКТИВНОГО ВІКУ З ПОРУШЕНЯМИ МЕНСТРУАЛЬНОГО ЦИКЛУ ПІСЛЯ ПЕРЕНЕСЕННОГО COVID-19

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Мета дослідження: вивчити структурно-метаболічні особливості будови ендометрія у жінок репродуктивного віку з порушеннями менструального циклу (ПМЦ) після перенесеного COVID-19, визначити кореляцію між гістологічним статусом та параметрами морфометрії ендометрія у даного контингенту пацієнтів.

Матеріали та методи. В роботі були застосовані бібліографічний, інформаційно-аналітичний, клініко-інструментальні, лабораторні, гістоморфологічні методи. Джерелами інформації були дані наукової літератури за темою дослідження, сучасні галлайни, огляд рандомізованих контролюваних досліджень.

Результати. Порушення менструального циклу по типу менометрорагії – один з основних мотивів звернення жінки до лікаря та госпіталізації. Серед причин розвитку ПМЦ по типу менометрорагії істотна роль належить порушенням гормонального гомеостазу зі зміною росту та диференціювання клітинних елементів ендометрія. Результати дослідження показали, що гістологічні структури ендометрію мають свою особливість у жінок репродуктивного віку з порушеннями менструального циклу після перенесеного COVID-19.

Висновки. Термін спостереження стану ендометрія у жінок репродуктивного віку з порушеннями менструального циклу після перенесеного COVID-19 може складати від 3 до 6 міс після перенесеного COVID-19. У жінок репродуктивного віку з порушеннями менструального циклу після перенесеного COVID-19 гіперпластичні процеси не спостерігаються.

Ключові слова: менометрорагія, гіперпластичні процеси ендометрія, порушення менструального циклу, пандемія COVID-19, психологічний дистрес.