Pathomorphological Analysis of Internal Endometriosis

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We performed clinical and morphological examination of 59 women with internal endometriosis (adenomyosis). Women were found to develop adenomyosis more frequently in the perimenopausal period (in their 40s) after repeated abortions and diagnostic surgical procedures. In 90% patients, adenomyosis was associated with the formation of multiple leyomyomatous nodules; glandular hyperplasia of the endometrium and fibrocystic transformation or fibrous degeneration of the ovaries were found in 60 and 100% cases. Two morphological variants of adenomyosis were identified: invasion of cytogenic stroma into the underlying myometrium with the formation of endometrial glands and invasion of cytogenic stroma via connective tissue interlayers without formation of endometrial glands and with pronounced neoangiogenesis. Foci of active adenomyosis in the uterus with predominance of cytogenic stroma were most prevalent in the late reproductive period. Endometrioid heterotopias were accompanied by considerable structural and functional reorganization of the myometrium with the formation of multiple leyomyomatous nodules. The presence of active and inactive adenomyosis foci and leyomyomatous nodules in uterus specimens reflects their metachronous development.

Key Words: *adenomyosis; clinical manifestations; pathomorphology*

Endometriosis (EM) is a chronic disease causing major disturbances of female reproductive function, infertility, and disability [1,8]. Conservative treatment of EM is usually little effective and produces only transient clinical effect, which makes EM one of the major unresolved problems in modern gynecology [3,6]. Although there are many studies on the etiology and pathogenesis of EM, these issues remain unresolved and controversial. Genital EM is commonly subdivided into internal (adenomyosis) and external (ovarian endometriosis), which often do not coincide. Since significant morphogenetic, pathogenetic, and molecular biological peculiarities of adenomyosis and external EM were identified, some researchers consider these clinical and morphological variants as individual nosological units [3,4,10].

Differential approach to elucidation of the pathogenesis of various clinical and morphological genital EM variants is necessary. It requires the search for new methods of treatment of adenomyosis and ovarian EM, if not achieving full recovery, but at least slows the rate of structural and functional changes in the uterus and ovaries. In this respect, clarification of the major factors (genetic, epigenetic, medical, and social) causing the development of different variants of EM is of particular importance [3,11]. Morphological methods play a key role both in the diagnosis of EM and also in establishing the basic morphogenetic processes leading to the formation of endometrial structures outside the endometrium (endometrioid heterotopias). Morphological assay clarifies the activity of the morphogenetic transformations, which has important prognostic value and determines the treatment. Morphological analysis is also important for systematization and classification of endometrioid heterotopias andverification of hypotheses on the origin and development of EM.

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Currently, three major concepts of EM development are regarded by most researchers: *in situ* formation of EM from embryonic remnant structures; induced metaplasia as a result of differentiation of mesenchymal cells; hematogenous or lymphogenous dissemination of endometrial cells followed by their implantation [3]. The major morphogenetic events of adenomyosis are penetration (invasion) of glandular and stromal components of the endometrium into the underlying myometrium and considerable modification of the myometrium stroma facilitating the spread of endometrium heterotopy. The development of external genital and extragenital EM is associated with retrograde spread of endometrial structures (functional layer of the endometrium) shed during menstruation, and the development of adenomyosis, exclusively with the invading components of the endometrial basal layer.

Here we made a pathological analysis of adenomyosis identifying its main morphogenetic variants.

MATERIALS AND METHODS

Fifty-nine women 42-74 years old (mean age 50.1±0.7 years) with adenomyosis were examined by means of clinical and morphological methods in the Municipal Novosibirsk perinatal center in 2007-2011 years. In the preoperative period, hormone therapy for cystic glandular hyperplasia of the endometrium and endometriosis was carried out in 10 (17%) women. No clinical effect was achieved, therefore surgical treatment corresponding to the severity of the disease was recommended to all patients. In women of reproductive age, the uterus without adnexa was supracervically amputated in order to maintain hormonal levels. The patients in the menopausal period were subjected to hysterectomy with adnexa. Before hysteroscopy, laparotomy was performed in all patients, and in most cases, separate diagnostic curettage of the uterine cavity and cervical canal was performed in order to exclude malignant growth.

Samples of the uteri amputated during surgery and resected ovaries were fixed in 10% neutral formalin, stained with hematoxylin and eosin and after van Gieson, and analyzed under Leica DM 4000B universal microscope using Leica DFC 320 digital camera and software Leica QWin.

RESULTS

According to our observations, adenomyosis was diagnosed mainly in perimenopausal females: 80%, in premenopausal women; 17%, in menopausal women; 3%, in postmenopausal women. Clinical and instrumental investigations showed that in 93% cases adenomyosis was accompanied by multiple interstitial uterine leiomyomas (predominantly with multiple interstitial ones, less frequently, submucous and subserous). In assessing the duration of adenomyosis, we relied on the duration of uterine myoma, which averaged 7.94±0.97 years (from 1 year to 23 years). True duration of adenomyosis, as well as its frequency, cannot be found at this stage of the research because of limited diagnostic capabilities. Adenomyosis combined with multiple uterine fibroids manifested in dragging pain in the abdomen, spotting from the genital tract before and after menses, heavy and prolonged menstrual bleeding leading to anemia; in the menopausal period, bleeding from the genital tract, and dysuria. In 7 (12%) females, foci of adenomyosis were casually detected by ultrasonography during preventive examination.

Uteri removed due to combined adenomyosis and uterine leiomyomas, were usually spherical and enlarged (up to 6-15 weeks of gestation) mainly due to thickening of the muscular layer. In 62% cases, adenomyosis was accompanied by glandular endometrial hyperplasia (mostly simple hyperplasia of endometrial glands). In the samples of removed uterus, eutopic endometrium was presented by cytogenic stroma, in which endometrial glands of secretory type were located. In some cases, cystic transformation of the glands was observed during glandular endometrial hyperplasia. In such formations, the epithelium was low, mainly cubic; the lumen of the glands contained a flocculent substance. Endometrioid heterotopia manifested itself in two morphological variants: ingrowth (invasion) of cytogenic stroma into the myometrium with the formation of endometrial glands of secretory type (Fig. 1, *a*), and ingrowth of cytogenic stroma along the vessels into the myometrium with the formation of endometriotic foci of various sizes without glandular structures (Fig. 1, *b*).

Foci of adenomyosis are usually analyzed according to their extent and morphofunctional characterization. In evaluating the morphofunctional state of adenomyosis, two trends in the evolution of foci were distinguished: progression and regression, or active and inactive foci [2,10]. In progressive (active) foci, secretory changes of the glands were noted as well as proliferation of the glandular epithelium, and sometimes decidualization of cytogenic stroma. Regressing (inactive) foci of adenomyosis were characterized by cystic transformation of the glands, and flattening (metaplasia) of the epithelium. When assessing active and inactive adenomyosis, some authors identify the relationship of cytogenic stroma and glandular epithelium as the main morphological criteria: adenomyosis foci with predominance of cytogenic stroma are classified as active, and those with poorly developed stroma and dystrophic changes in the epithelium, as inactive ones [9]. In our observations, foci of progressive (ac-

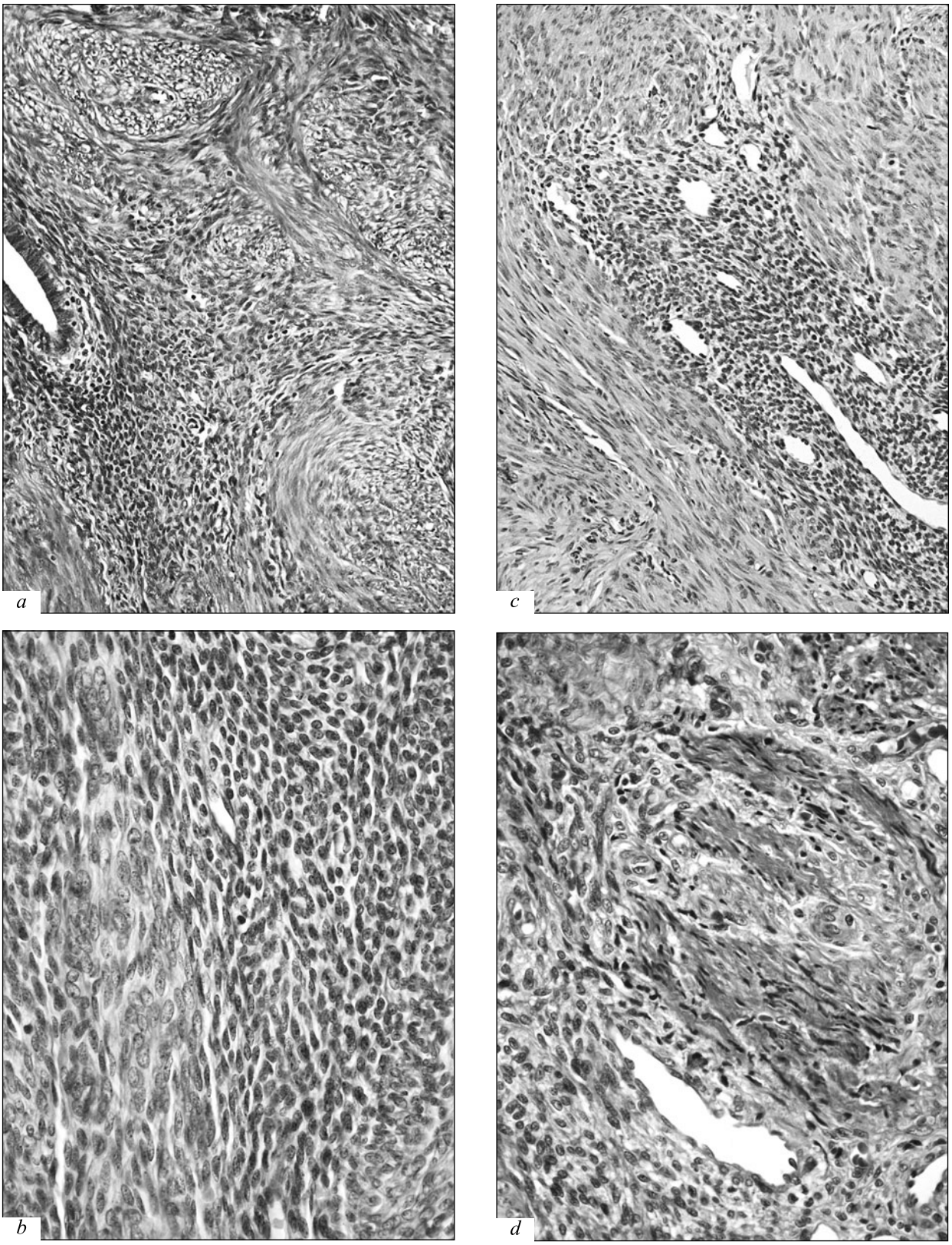

Fig. 1. Morphological variants of adenomyosis. Hematoxylin and eosin staining. *a*) extension (invasion) of cytogenic stroma into the myometrium with the formation of endometrial glands; impaired architecture of the myometrium due to the formation of leyomyomatous nodules and random orientation of leyomyocytes (×200); *b*) extension of cytogenic stroma into the myometrium without the formation of endometrial glands; active neoangiogenesis (×400); *c*) focus of adenomyosis with blood vessels (×200); *d*) leyomyomatous nodule surrounded with cytogenic stroma; heterogeneous leyomyocytes (×400).

tive) adenomyosis dominated, with the prevailing cytogenic stroma and secretory glands characteristic for endometrial proliferative phase; regressing (inactive) foci were less common. Diffuse and focal and diffuse forms of adenomyosis II-III degree prevailed.

It should be noted that this approach is appropriate for the morphological variants of adenomyosis with the formation of endometrial glands. In evaluating the morphofunctional state of adenomyosis foci formed by cytogenic stroma, they can be classified as progressive (active) or regressive (inactive) according to the extent of neoangiogenesis. According to our observations, cytogenic endometrial aggression was always associated with enhanced neoangiogenesis, formation of small blood vessels (capillaries, arterioles and veins of sinusoidal type) in the foci of endometrioid heterotopias. By the morphogenetic potential, it resembled malignant proliferative process (in particular, the sarcomatous invasion of carcinosarcoma). Some authors have noted that the stromal component is preferably subjected to malignancy during neoplastic transformation of adenomyosis [12], but the others refer endometrial stromal sarcoma to rarely found neoplastic processes in the uterus [15].

Progression (activity) cytogenic endometriotic foci were specified according to the phases of neoangiogenesis (clusters of endothelial-like cells, formation of vascular buds without the expressed lumen, emergence of vascular buds with apparent lumen, capillaries and small vessels with erythrocytes, formation of large sinusoid-like vessels). Foci of adenomyosis with clusters of endothelial-like cells, vessel buds and chains as well as numerous capillaries full of blood were referred to the active ones (progressive; Fig. 1, *b*), and the foci with larger sinusoid-like, to inactive ones (silent; Fig. 1, *c*). However, such a classification is relative since morphogenetic transformations occur constantly during adenomyosis. In tested samples the active foci of "cytogenic-stromal" adenomyosis varying in size dominated, spreading diffusely along the myometrium.

The prevalence of active foci of adenomyosis ("glandular" or "stromal") in our observations is probably due to the fact that the most surveyed women were of late reproductive age (premenopausal), when hormonal regulation of the functional activity of the endometrium, in particular, proliferation and differentiation of endometrial cells is preserved, albeit impaired. The lack of dynamic data observing adenomyosis foci does not allow us to make informed conclusions whether different morphological variants of adenomyosis ("glandular" and "stromal") are stages of the same process or they are relatively independent morphogenetic forms. However, we considered prevailed cytogenic stroma in adenomyosis foci combined with neoangiogenesis as unfavorable prognostic criteria of the evolution of endometrioid heterotopias.

Considerable structural and functional reorganization (remodeling) of the myometrium is one of the most severe consequences of endometrioid heterotopias in the uterus. This process is always of prevalent transmural nature. When adenomyosis was combined with leiomyomas, numerous nodules of leyomyocytes were always detected both in the myometrium and near eutopic and ectopic endometrium, surrounded by strands of smooth muscle cells and stroma (Fig. 1, *a*). In the adjacent myomatous nodules, leyomyocytes were irregularly arranged. They formed vortex-like strands thus strongly impairing architectonics of the myometrium, especially in subendometrial layer, where these processes were the most pronounced. Two types of leyomyomatous nodules were observed, with active hyperplasia of leyomyocytes (progressive growth) and dystrophic changes in leyomyocytes (regressive growth). In the latters, smooth muscle cells showed pronounced polymorphism, vacuolation and patchy "devastation" of their sarcoplasm (Fig. 1, *d*). In some leyomyomatous nodules, atypia of the nuclei of smooth muscle cells was observed (strongly hyperchromatic, elongated, often convoluted nuclei). In most investigated cases, the combination of progressive and regressive leyomyomatous nodules were recorded indicating asynchronous development, probably due to wavy dyshormonal shifts. Leyomyomatous transformation of myometrium usually dominated and was accompanied by considerable fibrosis (intercellular and internodular).

In all cases, adenomyosis was associated with significant structural and functional changes in the ovaries: in 67% cases, formation of multiple follicular cysts and ovarian fibrosis, in 33%, ovarian fibrous degeneration. These morphological changes reflected the apparent dysfunction of the ovaries during adenomyosis, which could be another cause of impaired uterine morphogenesis. Perhaps the expression of aromatase cytochrome P-450 in eutopic and ectopic endometrium at varied localization of endometriosis is not only involved in the pathogenesis of this disease [7], but reflects the compensatory-adaptive responses aimed at maintaining the estrogen level during ovarian dysfunction.

The fact that we revealed adenomyosis predominantly in perimenopausal women agrees with the results of other researchers [1] who reported adenomyosis to be diagnosed more frequently in the older women of reproductive age (40.1% cases), and in premenopausal women (32%), but considerably less, in postmenopausal women (15%). Among the factors provoking the development of adenomyosis are particularly intra-uterine interventions (repeated curet-

tage, manual examination of the uterus, *etc*.). These procedures promote the destruction of the morphological barrier between basal layer of endometrium, and myometrium [1]. We have found medical abortions in obstetric history of 95% patients while 38% women were subjected to 5 or more abortions (in half of the cases, up to 8-15). In addition, in 71% women separate diagnostic curettage of the cervical canal and the walls of the uterus was performed at different times before the surgery.

The main causes initiating endometrioid heterotopias and leyomyomatous transformation of the myometrium in this group of patients can be repeated damage of the endometrium/myometrium and imbalances in steroid (including sex) hormone levels in women after medical abortion. Hormonal imbalance caused by the interruption of pregnancy, and age-related dyshormonal shifts may result in impairments in regulation of proliferation and differentiation during reparative regeneration of endometrium/myometrium. A large (deep) alteration of the endometrium during medical abortions is associated with increased levels of proinflammatory cytokines, many of which have mitogenic and differentiation activity (*i.e*., as growth factors), and increased expression of metalloproteinases [4,5,11,14]. All this leads to the modification of extracellular matrix, facilitates invasion and migration of epithelial and stromal cells of endometrium into myometrium, and causes ectopic histogenesis. Genetic factors determining the severity of violations of the hormonal system, expression of proinflammatory and antiinflammatory cytokines, matrix metalloproteinases, adhesion and chemotactic molecules certainly make a significant contribution to the endometrial ectopia [13].

Thus, invasion of endometrial stromal cells into the myometrium plays a leading role in the formation of heterotopic endometrial foci in the uterus. Migration capacity (the causes of this phenomenon) still remain unclear, provides their penetration via connective tissue interlayers (mainly along the blood vessels) into the myometrium. In premenopausal period, active foci of adenomyosis are formed more frequently due to high proliferative activity of stromal endometrial cells. Morphogenetic potential of stromal cells is manifested in their capacity to induce the formation of endometrial glands and blood vessels, hyperplasia of smooth muscle cells, and formation of leyomyomatous nodules, probably due to synthesis of paracrine regulatory factors. This substantiates the need of detailed study of the biology of endometrial multipotent stromal cells aimed to development of new methods for regulation of their mitogenic and migration activity.

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