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Possibilities of Ovarian Endometriosis Neoplastic Transformation

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ABSTRACT

Objective: to analyze the signs of neoplastic transformation of the epithelium of ovarian endometriosis foci.
Materials and methods: We have analyzed 78 and 35 cases of ovarian endometriosis, using the histological and immunohistochemical methods respectively, and 8 cases of adenocarcinoma. The analysis took into account antibodies to Ki-67, Bcl-2, p53 and hepatocyte nuclear factor-1beta (HNF-1β).
Results: In the epithelium of endometrioid cysts walls, we determined syncytial papillary changes (39.7%), hobnail cell metaplasia (15.4%), and epithelium atypia (41.0%) with the expression of Ki-67 and Bcl-2 fluctuating from low to relatively high values and with the expression of p53 with a tendency to increase. HNF-1β expression in the foci without atypia was detected in 56.3% of cases, and in the foci with atypia - in 94.7% of cases; it was found only in clear cell adenocarcinomas.
Conclusion: HNF-1β hyperexpression suggests the adaptive nature and histogenetic connection of ovarian endometriosis with clear cell ovarian tumors.

KEYWORDS

Ovarian Endometriosis, Atypical Ovarian Endometriosis, Hepatocyte Nuclear Factor-1beta (HNF-1β), Clear Cell Ovarian Adenocarcinoma.

Introduction

Endometriosis is one of the most “topical” diseases in modern gynecology and represents a multi-factorial state, characterized by accrementition of a tissue, in morphologic and functional properties similar to endometrium, outside the uterine cavity. This disease has some characteristics of a malignant process: presence of local and distant foci, the ability of cells to migrate, invasion followed by damage to target organs [1,2].

The histogenesis of ovarian endometriosis (OE) and endometriosis-associated tumors still remains a topic of discussion. For the patients with OE, the risk of malignant ovarian neoplasms increases 2.5 times [3]. 12.1% of OE patients have epithelial metaplasia in endometriosis foci, 9.4% - hyperplasia, and 5.9% - atypia; and 4.1% of such patients are diagnosed with ovarian cancer [4]. According to other authors, the incidence of epithelial atypia in OE foci reaches 35-80% [5].

Ovarian cancer is a heterogeneous group of tumors in morphologic and molecular-biological terms. Mutation of p53 gene is typical for serous tumors, mutation of K-Ras - for mucinosis tumors, and mutations of β-catenin and PTEN - for endometrioid tumors. The hepatocytes nuclear factor-1beta (HNF-1beta or HNF-1β) is recognized as a specific immunohistochemical (IHC) marker of clear cell ovarian tumors, which is most often associated with OE. Other ovarian and endometrial tumors do not express HNF-1β [6,7,8]. HNF-1β is a transcription factor superfamily protein that binds to DNA as a homo- or heterodimer and normally participates in pancreatic and nephron embryogenesis. Mutations in the gene coding this transcription factor were found in polycystic kidney disease, some types of diabetes mellitus, and some tumors (of ovaries, endometrium, prostate, and kidneys). HNF-1β plays an important role in the mechanisms of cell protection against oxidative stress, inhibits apoptosis by interacting with the apoptosis inhibitor Bcl-2 [6,7,8]. It was shown that HNF-1β hyperexpression in clear cell tumors underlies their resistance to chemotherapy. The role of HNF-1β in the mechanism of epithelium neoplastic transformation in OE foci is also discussed. Its expression was revealed in the epithelium of 40% of endometrioid ovarian cysts and is more often observed in the epithelium atypia of endometriosis foci [6,7,8,9,10,11].

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The objective of this research was to study the morphological and molecular-biological signs, which indicate potential neoplastic transformation of the epithelium of OE foci, in order to reduce the recurrence of ovarian endometriosis and to preserve fertility in women of reproductive age.

Materials and Methods

We have analyzed archival materials (dated 2014-2016) from the A.K. Yeramishantsev City Clinical Hospital and the Bakhrushins' brothers City Clinical Hospital. The materials were the case histories of 147 patients of reproductive period with confirmed diagnosis "ovarian endometriosis" or "recurrent ovarian endometriosis". The age of the patients was from 18 to 45 years with the prevalence of the range from 27 to 35 years. The average age of the patients was 32.1 years.

The data of medical cases were divided into 3 main groups (group 1 – patients with pelvic pain, group 2 – patients with infertility, group 3- patients with menstrual disorders). The gynecological and somatic anamneses were analyzed to detect the patients with previously established diagnosis of OE and surgical treatment. We received the results of laboratory research methods (including oncomarkers and AMH levels) and defined the main patterns in the descriptions of pelvic ultrasound examinations (PUS), which were submitted before admission for surgical treatment in the hospitals.

After ovarian resection for OE, we obtained 147 histological samples in the examined group of patients; 78 fragments of ovarian tissue were selected for further detailed study. At that, the study included 8 ovarian adenocarcinomas (two cases of clear cell, two cases of well-differentiated serous, two cases of endometrioid and two cases of mucinous ones). OE was not identified in the medical histories and surgical materials of patients with adenocarcinomas.

In the histological examination and study of the selected observations, we used the standard method of fixation in 10% neutral formalin, paraffin embedding, made histological sections of 3 μm thick paraffin blocks using an HM355S rotary microtome (Thermo Scientific, Germany) - straightened on slides and subsequently stained with hematoxylin and eosin. The examination of samples confirmed and clarified the histopathological diagnosis, identified endometriosis foci of in the ovarian tissue in addition to the endometrioid cyst; it also allowed selection of paraffin blocks for immunohistochemical (IHC) studies. OE was confirmed on the basis of generally accepted morphologic criteria: the presence of the epithelial lining of the cyst and/or glandular structures from endometrioid cells (taking into account reactive, regenerative, dystrophic, metaplastic changes and atypia), the presence of endometrioid stroma and macrophages with hemosiderin in the infiltrate and / or cyst lumen [12,13,14].

The immunohistochemical method was used to study 35 observations with OE (examined with four mono- and polyclonal antibodies) and 8 observations with ovarian adenocarcinomas (examined only with polyclonal antibodies to HNF-1β). The primary antibodies were antibodies to proliferating cells marker, the nuclear protein Ki-67 (monoclonal antibody, Clone MIB-1, DAKO, Norway, diluted 1: 150), the apoptosis inhibitor Bcl-2 (monoclonal antibody, Clone 124, Cell Marque, USA, diluted 1: 250), p53 oncoprotein (monoclonal antibody, Clone DO7, Cell Marque, USA, ready-to-use dilution), transcription factor superfamily protein, hepatocyte nuclear factor -1beta (HNF-1β, polyclonal antibody, GeneTex, USA, diluted 1:200).

The reactions were performed simultaneously for the whole material, in accordance with the methodology of S.R. Shi et al. and according to the manufacturers protocols attached to mono- and polyclonal antibodies [15,16]. The visualization of reactions was done with a ready-to-use test system with universal secondary antibodies labeled with chromogen (3,3'-diaminobenzidine) - Histophine (Nichirei Corp., Japan). The method of Histochemical score (H-score) [17] was used to evaluate the results of IHC reaction; the number and intensity of the stained cell nuclei (Ki-67, p53, HNF-1β) and cytoplasm (Bcl-2) were taken into account as follows: up to 80 - low, 80-140 - moderate, 141-300 - high expression. Counting was performed for 100 epithelial cells in 3 randomly selected fields of vision at the x400 microscope magnification. The results were statistically processed using MS Office Excel and Statistica for Windows 10.0 software, and presented as "average value ± standard square deviation (M ± σ)"; the results were subjected to Mann-Whitney non-parametric analysis. The value of the significance critical level was assumed to be 0.05.

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The patients with OE were subsequently monitored in hospitals, in order to check the effectiveness of surgical treatment and get or correct necessary therapy after obtaining the results of a histological examination; the therapy was aimed at reducing the risk of possible endometriosis recurrence and preserving the reproductive period patients' fertility. The level of oncomarkers (CA-125, CA-19-9, CA-72-4, CA-15-3) and the level of AMH were monitored with pelvic ultrasound examination - 6 months and 1 year after the surgical treatment. The patients were also prescribed gonadotropin-releasing hormone agonists (aGnRH) for 3 months, and, in the absence of the patient's desire to bear a child, progestin group drugs for 6 months.

Results

In 73.5% of the cases, the examined patients had complaints on admission. The most frequent of them were infertility (43.54% of the cases) and pelvic pain (36.73% of the cases); the less frequent complaints were menstrual disorders (14.97% of the cases).

10.2% of all patients had underwent OE-related surgical treatment; 1.4% of the patients had underwent repeated surgeries related to OE relapse. The time intervals from the original surgeries to OE recurrence in the 10.2% of cases (the surgical treatment was performed in the A.K. Yeramishantsev City Clinical Hospital and the Bakhrushins' brothers Clinical Hospital) were: 0.25 years (3 months) in one patient, 1 year in three patients, 2 years in three patients, 3 years in one patient, 4 years in four patients, 5 years in one patient, 6 years in one patient, 8 years in one patient. In the 1.4% of cases (two women), the time intervals from the primary acute condition treated by a surgery till the repeated surgeries performed prior admission to the Hospitals were 4 months and 72 months. Later, only 17.65% of the patients were prescribed medication hormonal therapy.

Burdened gynecological and somatic anamneses in the examined patients were observed in 48.3% and 51.7% of cases respectively. The most frequent diseases were cervical ectopy (44.44%), chronic salpingo-oophoritis (26.39%), uterine myoma (22.22%), adenomyosis (22.22%), as well as gastrointestinal diseases (71.05%), cardiovascular diseases (21.05%) and respiratory diseases (11,84%).

All the patients had the results of full laboratory tests and instrumental examinations performed in antenatal clinics before entering the Bakhrushins' brothers and Yeramishantsev Hospitals. The assessment of the level of oncomarkers, such as CA-19-9, CA-125, CA-72-4, HE-4, CEA before admission to the Hospitals, is given in Table 1.

Table 1. Characteristics of diagnostic on co-markers in patients prior to surgery

Oncomarker (OM)	**Normal range (U/ml)**	**No. of patients assessed**	**Variation (U/ml)**	**No. of patients with increased OM levels (%)**	**Mean levels of increased values**
CA-125	0-35	74 (50.34%)	2-273	36 (48.65%)	77.90±8.35
CA-19-9	0-37	51 (34.69%)	0.6-136	6 (11.76%)	80.53±12.37
CA 72-4	0-8.2	7 (4.76%)	1.69-35.9	4 (57.14%)	19.72±6.47
CA 15-3	0-31.4	16 (10.88%)	1-19.99	-	-
HE-4	0-140	7 (4.76%)	15.6-68.45	-	-
CEA	0-4.9	18 (12.24)	0.3-1.87	-	-

The level of AMH was examined in 2.04% of cases; all the indicators before surgical treatment were within reference values.

The pelvic ultrasound examination made before surgical treatment mainly described OE as a single-chamber unilateral formation with sizes from 3 to 6 cm (85.03% of cases) with anechogenic structure (59.18% of cases) with fine-dispersed contents (59.18% of cases). The walls of the capsule were described as well-defined and even in all cases and only in 6.80% of cases PUS showed their induration. The description of the ovarian formation blood flow existed only in 6.80% of cases; the formation was avascular in all these reports. Adhesive process in the small pelvis was indicated only in 6.80% of cases, and retrocervical endometriosis - in 1.36% of cases. Thus, according to the classification for endometrioid ovarian cysts (L.V. Adamyan, 1992, 1998), most of the above descriptions corresponded to stage II-III of the disease [18].

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Laparoscopic unilateral resection of the ovary within the affected tissues was performed in 82.99% of cases, while 12.24% of cases involved bilateral resection. Coagulation of variously localized endometriosis lesions was performed in 68.71% of cases, retrocervical endometriosis resection - in 1.36% of cases, and adhesiolysis - in 60.54% of cases.

During subsequent follow-up of patients with OE and control of AMH and oncomarker levels (CA-125, CA-19-9, CA-72-4, CA-15-3) it was revealed that all indicators were within the reference values. PUS examination showed a slight decrease in the size of the previously resected ovary, but the follicular apparatus was clearly visualized in sufficient quantity. In 6.80% of cases, a 1-2-stage adhesion process was recorded in the region of the operated adnexa.

It is important to mention that against the background of the conducted follow-up and the prescribed treatment, ovarian endometriosis relapse did not occur in any patient, which suggests adequate volume of surgical treatment and a correctly developed individual medication therapy. In 22.72% of cases among the patients with infertility, a spontaneous progressive pregnancy occurred during one year of follow-up, which was recorded by ultrasound examination.

In 43 out of 78 histologically studied OE observations (55.1%), histological tests revealed one to four endometriosis foci within the resected ovarian fragment with endometrioid cyst (represented by epithelial and stromal components), both in the walls of the cyst and at different distances from it.

Out of the 78 studied observations, in the epithelium of endometrioid cyst walls and / or other endometriosis foci, there were micro focuses or larger areas with syncytial papillary regenerative (hyperplastic) changes (31 cases, 39.7%), metaplasia with appearing hobnail cells (12 cases, 15.4%) and focal hyperplasia (papillary, glandular-solid, with areas of squamous metaplasia and epithelial atypia) (three cases, 3.9%). Epithelial atypia was detected in 32 cases (41.0%), where in 27 cases (34.6%) the atypia was regarded as "regenerative / dystrophic" one [12,13,14] due to marked dystrophy of epitheliocytes combined with accumulation of intraepithelial leukocytes and inflammatory infiltration of the adjacent stroma of the endometriosis lesion. In five cases (6.4%), these changes were absent and a true, "neoplastic" atypia was diagnosed. In one case (1.3%), a small focus of a mucinous borderline tumor was found in the wall of the endometrioid cyst.

The selected 35 OE cases and eight ovarian adenocarcinoma cases were subjected to immunohistochemical analysis, the results of which are presented in Table 2.

Table 2. Results of immunohistochemical analysis of endometriosis foci and ovarian adenocarcinomas (M±σ)

Measure	**Endometriosis (lesions without atypia) n=35[1]**	**Endometriosis (lesions with atypia) n=32[1]**	**Clear cell adenocarcinoma n=2[1]**	**Serous adenocarcinoma (high grade) n=2[1]**	**Endometrioid adenocarcinoma n=2[1]**	**Mucinous adenocarcinoma n=2[1]**
Ki-67	27±14	79±11[3]	-[2]	-	-	-
p53	12±10	16±12	-[2]	-	-	-
Bcl-2	43±11	121±27[3]	-[2]	-	-	-
HNF-1β	62±17	188±13[3]	195±29[3]	Very few	Very few	Very few

Note: [1]n=35 is the number of cases where in endometrioid lesions, only foci without atypia were identified (only in 3 cases out of 35, no endometrioid lesions with foci of epitelhium with atypia were found); n=32 is the number of cases where in endometrioid lesions, one or more foci with atypia, along with foci without atypia, were found; [2]not assessed; [3]$p<0.05$ compared to endometrioid lesions without atypia.

Thus, the expression of Ki-67 proliferative activity indicator in the epithelium of OE foci was highly variable. Its level ranged from low to relatively high, mainly in the foci with regenerative/dystrophic and true epithelial atypia, in which the expression of other studied antigens was also notably large. The expression of oncomarker p53 was low, but it also tended to increase in the foci with regenerative/dystrophic and true epithelial atypia (however, the trend was statistically unreliable). The expression level of apoptosis inhibitor Bcl-2 was different, but mainly higher in foci with regenerative/dystrophic and true epithelial atypia.

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It is important to note that, in contrast to the foci with atypia, the lesions with syncytial papillary regenerative (hyperplastic) changes, with hobnail cells metaplasia and focal hyperplasia (papillary, glandular-solid or with areas of squamous metaplasia) did not differ from the unchanged endometriosis-specific epithelium in the expression of Ki-67, p53 and Bcl-2.

The expression of the specific transcription factor for clear cell ovarian and endometrial tumors HNF-1β was noticed in the epithelial nuclei of most endometriosis foci (77.1% of all 35 studied cases). It was detected both in the nuclei of a part of the endometrioid cyst epithelial cells and in the epithelium of other foci of endometriosis (found in the walls of cysts and in the ovarian tissue distant from them), regardless of the presence of metaplastic or hyperplastic epithelial changes. The calculation of the number of morphologically different endometriosis foci in 35 observations showed absence of atypia lesions in 16 cases; the expression of HNF-1β was determined for nine of them (56.3%). The epithelium nuclei in the endometriosis foci with atypia (regenerative/dystrophic or true one - detected in 19 cases out of 32 observations) expressed HNF-1β in 94.7% of such foci (18 cases). In the mucinous borderline tumor detected in the endometrioid cyst wall in one observation, HNF-1β expression was absent. There were no differences (histological, molecular-biological) in endometriosis foci in the patients with OE relapse.

The HNF-1β expression, which was also analyzed in various ovarian adenocarcinomas, was found only in clear cell adenocarcinomas. These tumors had a noticeable expression of this marker by the nuclei of most tumor cells. In other adenocarcinomas, only single tumor cells had weak or moderate expression of HNF-1β.

Discussion

The results of this study confirmed high incidence of the histological and molecular-biological changes in OE foci, which indicate a higher risk of neoplastic transformation. OE foci with histological signs of epithelial atypia (regenerative/dystrophic in 34.6% of cases, and true atypia - in 6.4% of cases) were characterized by higher expression of proliferation marker Ki-67, apoptosis inhibitor marker Bcl-2, and specific transcription factor of clear cell ovarian tumors HNF-1β; in OE foci there was also a tendency to hyperexpression of oncomarker p53. Moreover, there were no differences in the increased expression of all the studied antigens in the foci of regenerative / dystrophic and true atypia. That may be connected with a high degree of subjectivity of their histological differentiation. The generally accepted morphologic criteria for their differentiation do not exist; the absence or presence of inflammatory infiltrate (intraepithelial leukocytes, lymphocytes) and dystrophic changes in epithelial cells cannot serve as an objective criterion [12,13,14]. In addition, no features of endometriosis foci were revealed in patients with OE recurrence.

The revealed differences in the expression of Ki-67 and Bcl-2 in the foci with and without epithelial atypia allow us to explain why some studies showed an increase in their expression, while others did not confirm their hyperexpression. Besides, the level of these markers in the endometrium and endometriosis foci is subject to changes depending on the menstrual cycle phase [19].

The study shows that the epithelium of OE foci, regardless of the presence of signs of its atypia, is characterized by hyperexpression of the specific transcription factor of clear cell ovarian and endometrial tumors HNF-1β, which is noted in 56.3% of foci without atypia and in 94.7% of foci with atypia. This proves the validity of the assumption that HNF-1β hyperexpression in the epithelium of OE foci is widespread and adaptive in nature [6,7,8]. The histogenetic relationship of OE with clear cell ovarian tumors, for which HNF-1β expression is a diagnostic marker, is also confirmed.

Conclusion

Ovarian endometriosis should be a subject for further molecular-biological and genetic studies. They will help not only to clarify its relationship with ovarian tumors, but also to solve the problem of the origin of endometriosis itself. This will give an opportunity to identify the groups of women of reproductive age, who are at risk for presumptive development of a neoplastic process, and to closely monitor these groups of patients, followed by required scope of surgical treatment.

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