Ovarian endometrioid carcinoma, cytomorphological, cytogenetical and immunocytochemical findings

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Summary. Cytomorphological, cytogenetical and immunocytochemical findings in ovarian endometrioid carcinoma are presented on the basis of scrapings from tumors removed during surgery in 4 women of different ages. Two cytogram variants of round-oval and prismatic cells were identified. High counts in both total nucleoli and their active forms (compact and transitional nucleolonemal) were detected, which may be indicative of a poor prognosis of the disease. Immunocytochemical study with mAb - cytokeratin 7, 20 and epithelial antigen (Ber-EP4) can be helpful in the differential diagnosis of OC morphological variants.

Key words: ovarian endometrioid carcinoma, cytomorphological, cytogenetical and immunocytochemical findings.

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Introduction

Ovarian carcinoma (OC) ranks number eight in the overall structure of cancer morbidity and number three among tumors of the female reproductive system. Consistently, high incidence of malignant ovarian tumors (15.1 per 100 thousand) is registered in Ukraine with a tendency to increase [13].

Rational treatment of ovarian tumors is known to be possible only with regard to morphological diagnosis and prevalence of neoplastic process [9, 10, 11]. Histology is the "gold standard" for OC morphological diagnosis. However, recently cytological method has been increasingly used to determine the process stage both in the process of patient examination and during surgery. The object of cytological study is free peritoneal fluid, abdominal lavage, puncture biopsy specimens of ovarian tumors, Douglas' pouch, lymph nodes, tumor imprints, etc. [3-5, 15, 16, 18, 22].

Diagnosis of ovarian tumors remains one of the most difficult issues of oncomorphology due to their extremely diverse histological structure. Epithelial ovarian tumors are more common in the clinical practice. Ovarian endometrioid carcinoma (OEC) is a histological form that belongs to the group of germinal epithelial tumors. It makes up about 20% of all ovarian cancers and about 5% of all ovarian neoplasms. OEC generally is diagnosed in women over age 50. The tumor is usually unilateral, cystic and solid with papillary projections on inner surface of cystic cavities, often associated with endometriosis (42%) or endometrial carcinoma (15-20%) [20]. According to WHO histological classification, «endometrioid tumors of the ovary are neoplasms having morphological signs of tumors that occur in the endometrium». Endometrioid type of ovarian carcinoma is extremely polymorphic. There are numerous histological variants: OEC with squamous metaplasia, OEC on the surface of the ovary, malignant endometrioid adenofibroma, secretory variant, oxyphilic type, ciliated cell type, cellular variant, neuroendocrine variant, Sertoli cell variant [6, 18].

Tushur K. et al. [21] compared the results of intraoperative imprint cytology of ovarian tumors with the results of histological examination and found a low

diagnostic efficacy (66.6%) of OEC cytological diagnosis against other histological types of tumors. However, literature review showed only a few works dedicated to OEC study at the cellular level [1, 2, 8].

Purpose. To study cytomorphological, cytogenetical and immunocytochemical characteristics of ovarian endometrioid carcinoma (OEC).

Materials and methods

The cellular composition of OEC cytological specimens obtained by scrapings from ovarian tumors removed in 4 patients (aged 41, 42, 46 and 75) was studied and analyzed. Specimens were stained by Pappenheim's method. Cytomorphological signs of OEC were studied according to specifically designed «Scheme of cytological signs for the formal assessment of qualitative and quantitative composition of ovarian tumor cells» that includes 47 signs. General cell characteristics (count, localization, size and shape) were determined by assessment of cytological specimens. The size of cells and their components were compared with the size of erythrocyte: small – 1.5 - 2 times larger, medium – 2 - 5 times larger and big - 5 and more times larger than erythrocyte. Cytoplasm characteristics (survival, tinctorial properties) were studied; their morphological features – vacuolization and granularity – were also taken into account. Nucleus and nucleoli characteristics (shape, size, outline, chromatin pattern, color grade) were evaluated. Background components were noted.

Cytological specimens were stained with silver nitrate to identify nucleolar organizer regions (NORs) of chromosomes according to the method of Howell W., Black D. [17]. Studies of basic variants of NOR chromosomes were performed in accordance with the classification [14] and operational scheme aimed at identification of morphological and functional types of nucleoli [12].

Immunocytochemical studies with mAb (Dako) – cytokeratin 7; 20 (CK7, CK20), and epithelial antigen (Ber-EP4) were performed. All studied were performed using light microscope Olympus CX 41. OEC diagnosis was verified by histological examination of the surgical specimens.

Results

A significant number of cells arranged in groups (61%) and separately were found in most OEC specimens. In two cases, the cells were round and oval in shape and in two other cases they were predominantly prismatic.

In two cases OEC specimens demonstrated relatively monomorphic small and medium-sized (80%) cells. Often they contained one round or oval nucleus with irregular, sharply defined outline. As for tinctorial properties, hyperchromic nuclei with uniform finely clumped chromatin pattern were predominant. Unevenly distributed chromatin with lumens was identified in the portion of cells (Fig. 2-3). Single and multiple small nucleoli with poorly defined outline were noted. The vast majority of cells (75%) had relatively abundant basophilic cytoplasm, which was slightly to moderately colored. In the most isolated cells the cytoplasm was visualized and blended into general background of the specimen. A small number of tumor cells with hypochromic nuclei and abundant pale cytoplasm were identified (Fig. 3). The cytoplasm of individual cells contains fine vacuoles (10 - 15%) or basophilic stippling (30%) (Fig. 2).

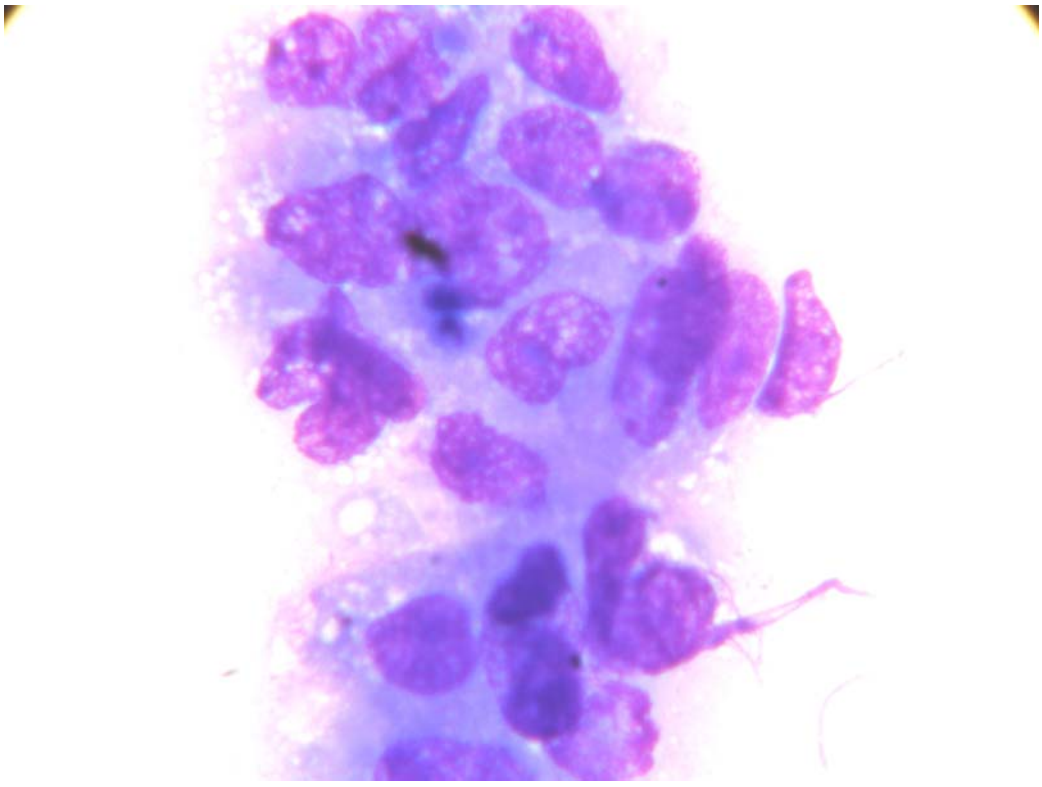

Fig. 1. The accumulation of mostly “bare” polymorphic nuclei with irregular chromatin pattern, individual cells with basophilic granular cytoplasm and globular

“lakes” of oxyphilic substance in OEC. Stained by Pappenheim. Magnification × 1000.

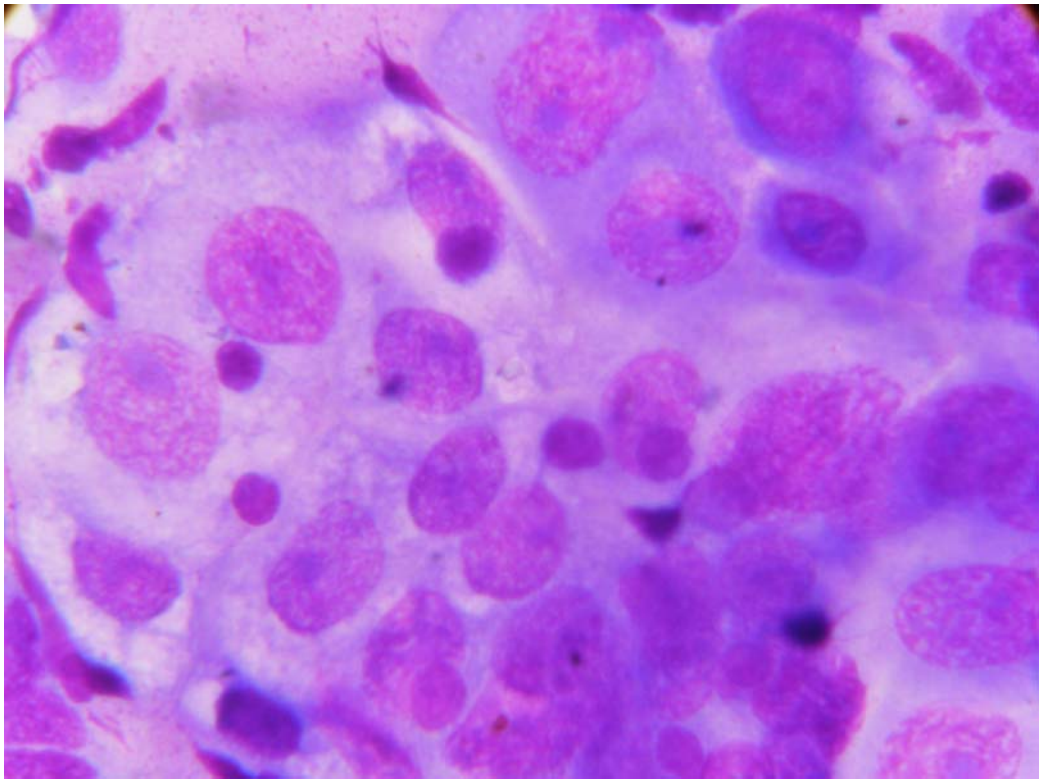

Fig. 2. The group of OEC tumor cells with polymorphic nuclei, irregular chromatin pattern and vacuolated cytoplasm. Stained by Pappenheim. Magnification × 1000.

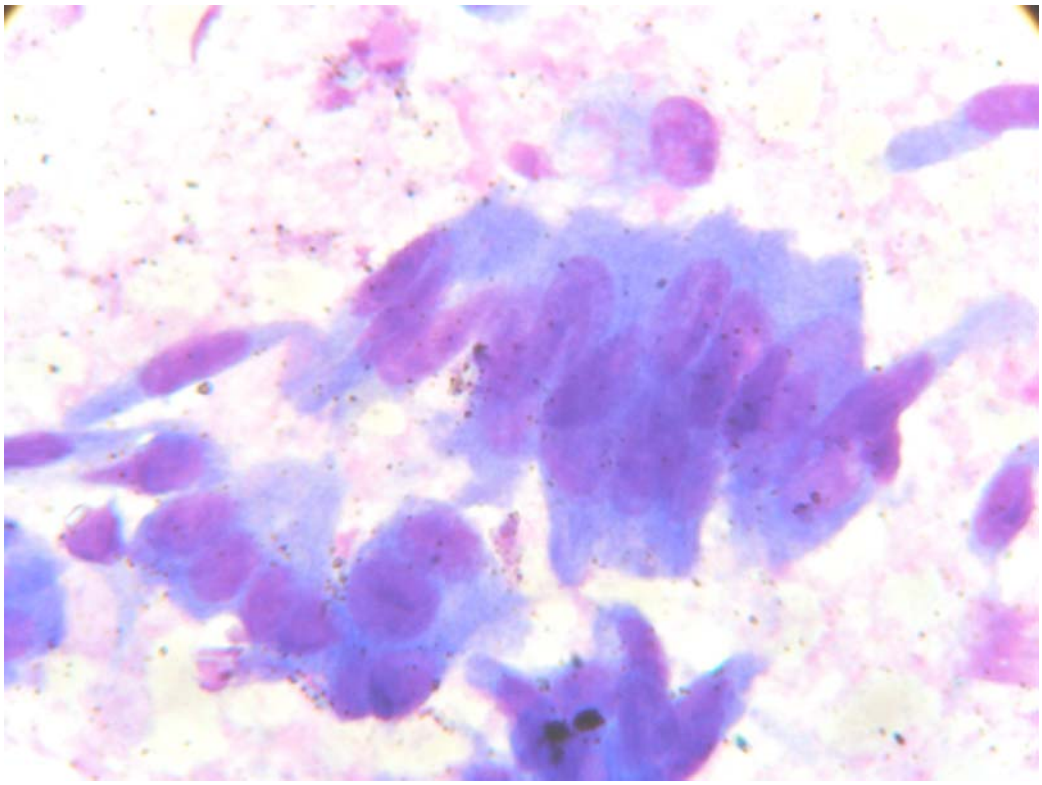

Fig. 3. The group of tumor cells with hypochromic nuclei and abundant pale cytoplasm. Stained by Pappenheim. Magnification × 1000.

A significant number of prismatic cell groups were identified in the specimens of two other patients (95%) (Fig. 4). Intensely colored tumor cells with minimal signs of atypia, parallel and closely adjacent to one another, resemble endometrial cells. The cytoplasm of tumor cells is elongated, intensely basophilic. The hyperchromic nuclei of prismatic cells are mainly arranged eccentrically (100%). Chromatin pattern of the most nuclei is uniform, finely stippled and clumped (40%), in isolated cases - compact. Nuclei with multiple polymorphic nucleoli with sharply defined outlines are predominant. There are layers of tumor cells that resemble “honeycombs”. In the middle of the layer cells are round with a thin band of intensely basophilic cytoplasm assuming elongated, prismatic shape at the margin. Similar patterns are characteristic of endometrial cells (Fig. 5).

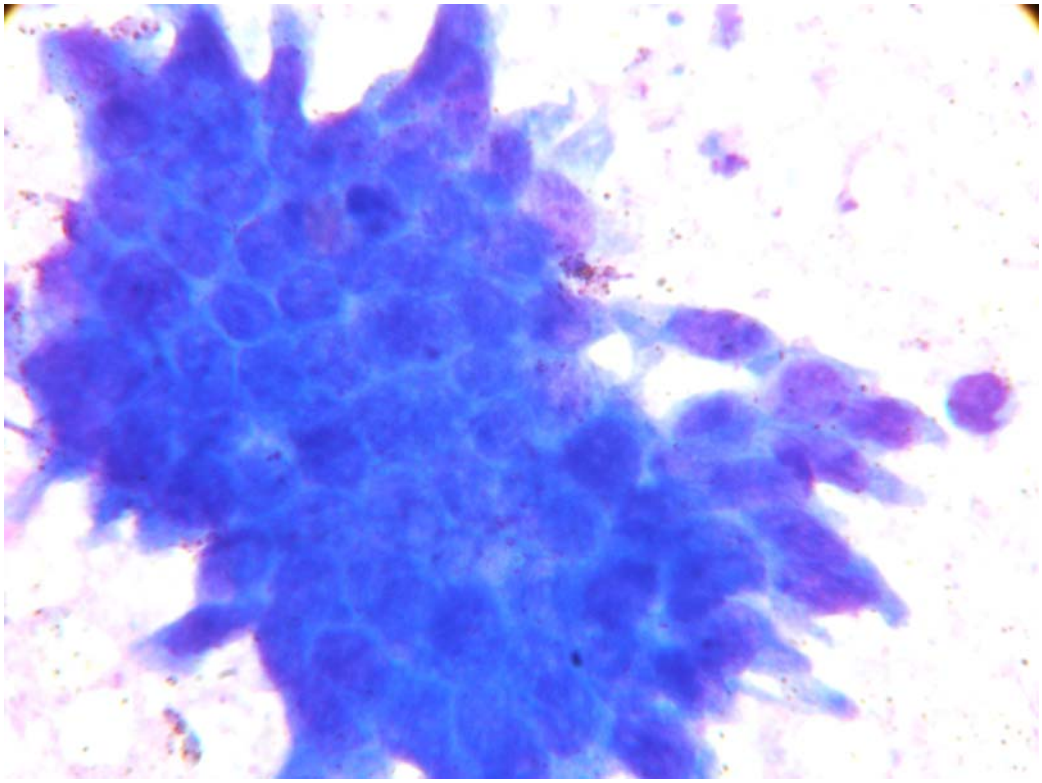

Fig. 4. Monomorphic highly prismatic and cuboidal OEC cells. Stained by Pappenheim. Magnification × 1000.

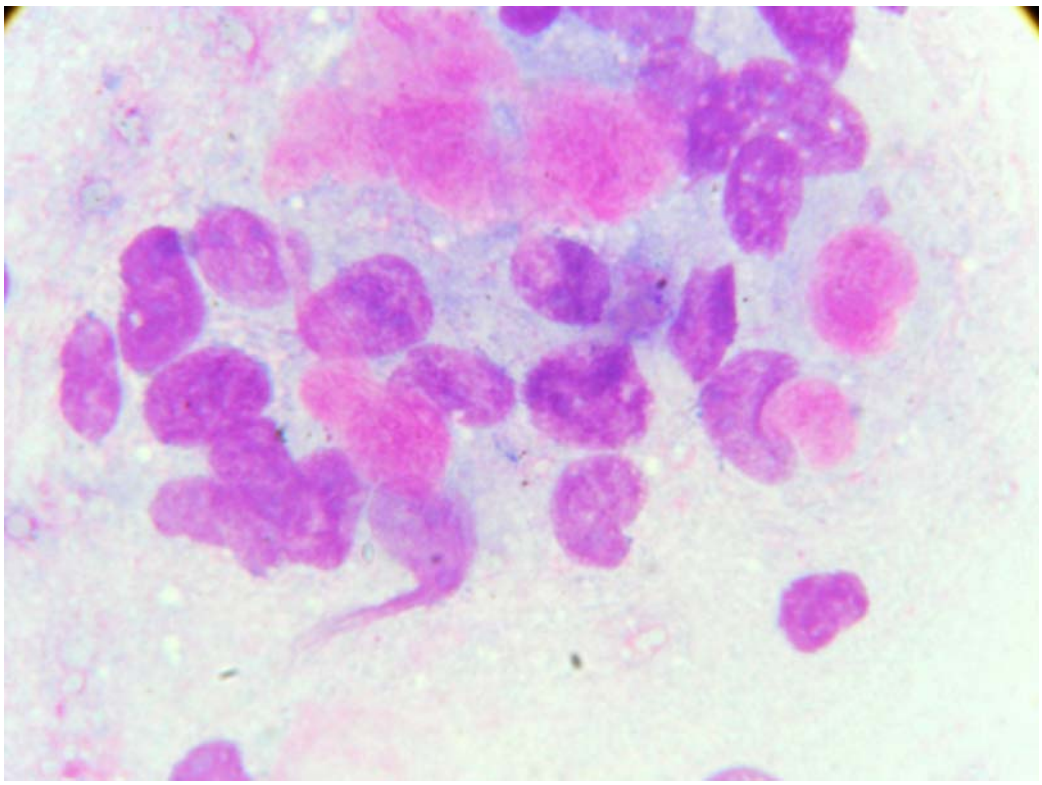

Fig. 5. The layer of tumor cells with hyperchromic nuclei and dense basophilic cytoplasm, resembling a “honeycomb”. Stained by Pappenheim. Magnification × 1000.

The study of OEC specimens revealed typical smear background, represented by oxyphilic masses. In each case amount and color of the mucoid substance differed from light-pink throughout the entire specimen in most cases to bright crimson (25%), which formed small or large “lakes”, located between the cells (Fig. 1).

In each OEC case qualitative typing of the basic types of nucleoli was performed in 100 tumor cell nuclei (Table).

Table. Basic morphofunctional types of nucleoli in OEC cells (M ± m), total, %, n = 4

№ п/п	Cell count	Compact nucleoli	Nucleolonemal nucleoli (absolute and transitional)	Ring-shaped nucleoli	Micro-nucleoli	Total count of basic types of nucleoli, average count in one nucleus
1.	Glandular, papillary endometrioid carcinoma *					
	100	54 0.54 ± 0.02 5.50 %	151 1.51 ± 0.02 15.50 %	196 1.96 ± 0.03 20.20 %	572 5.72 ± 0.07 58.80 %	973 9.73 ± 0.10
2.	Moderately differentiated endometrioid carcinoma *					
	100	53 0.53 ± 0.02 5.90 %	157 1.57 ± 0.02 17.40 %	178 1.78 ± 0.02 19.80 %	513 5.13 ± 0.08 56.90 %	901 9.01 ± 0.09
3.	Endometrioid carcinoma *					
	100	35 0.35 ± 0.01 4.82 %	150 1.50 ± 0.01 20.66%	150 1.50 ± 0.01 20.66 %	391 3.91 ± 0.03 53.86 %	726 7.26 ± 0.03
4	Endometrioid carcinoma *					
	100	30 0.30 ± 0.01. 5.18 %	149 1.49 ± 0.03 25.70%	89 0.89 ± 0.01 15.36%	312 3.12 ± 0.04 53.76 %	580 5.80 ± 0.04

* Histological diagnosis

In the specimens of patient T., diagnosed with glandular, papillary endometrioid carcinoma, total nucleoli count was 973; M = (9.73 ± 0.10). Compact nucleoli count was (0.54 ± 0.02) 5.50%. Average count of nucleolonemal nucleoli in the nucleus was (1.51 ± 0.02) 15.50%. Nucleolonemal nucleoli were represented only by transitional forms (100.0%). Underactive ring-shaped nucleoli

count was (1.96 ± 0.03) 20.20%, and inactive micronucleoli count was (5.72 ± 0.06) 58.80%.

In the specimens of patient B., diagnosed with moderately differentiated endometrioid carcinoma, 901 nucleoli were found; M = (9.01 ± 0.09). Average compact nucleoli count was (0.53 ± 0.02) 5.90%. Average count of nucleolonemal nucleoli in the nucleus was (1.57 ± 0.02) 17.40%. Among them transitional nucleoli accounted for 100.0%. Underactive ring-shaped nucleoli count was (1.78 ± 0.02) 19.80%, and inactive micronucleoli count was (5.13 ± 0.08) 56.90 %.

In the specimens of patient R., diagnosed with endometrioid carcinoma, 580 nucleoli were found, M = (5.80 ±0.04). Compact nucleoli count was (0.30 ± 0.01) 5.18%. Average nucleolonemal nucleoli count was (1,49 ± 0,03) 25,70 %, among them absolute nucleolonemal nucleoli accounted for 6.04%, and their transitional forms accounted for 93, 96%. Ring-shaped nucleoli count was (0.89 ± 0.01) 15.36%, and inactive micronucleoli count was (3.12 ± 0.04) 53.76 %.

In the specimens of patient L., diagnosed with endometrioid carcinoma, 726 nucleoli in 100 nuclei were found, M = (7.26 ± 0.03). Active compact nucleoli count was (0.35 ± 0.01) 4.82%. Average nucleolonemal nucleoli count was (1.50 ± 0.01) 20.66%. Among them transitional nucleoli percentage accounted for 100.0%. Underactive ring-shaped nucleoli count was (1.50 ± 0.01) 20.66%, and inactive micronucleoli count was (3.91 ± 0.03) 53.86 % (Fig. 6-7).

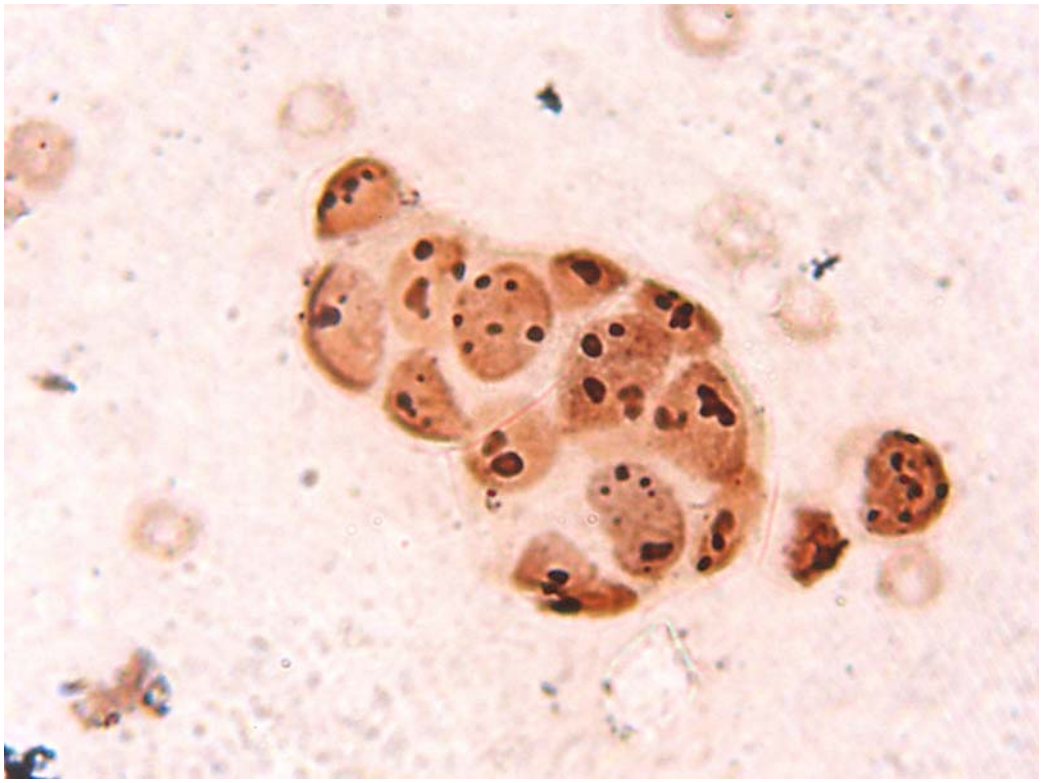

Fig. 6. The basic morphological types of nucleoli, predominantly compact, in the cells of moderately differentiated endometrioid carcinoma of the ovary. Stained by Howell W., Black D. Magnification × 1000.

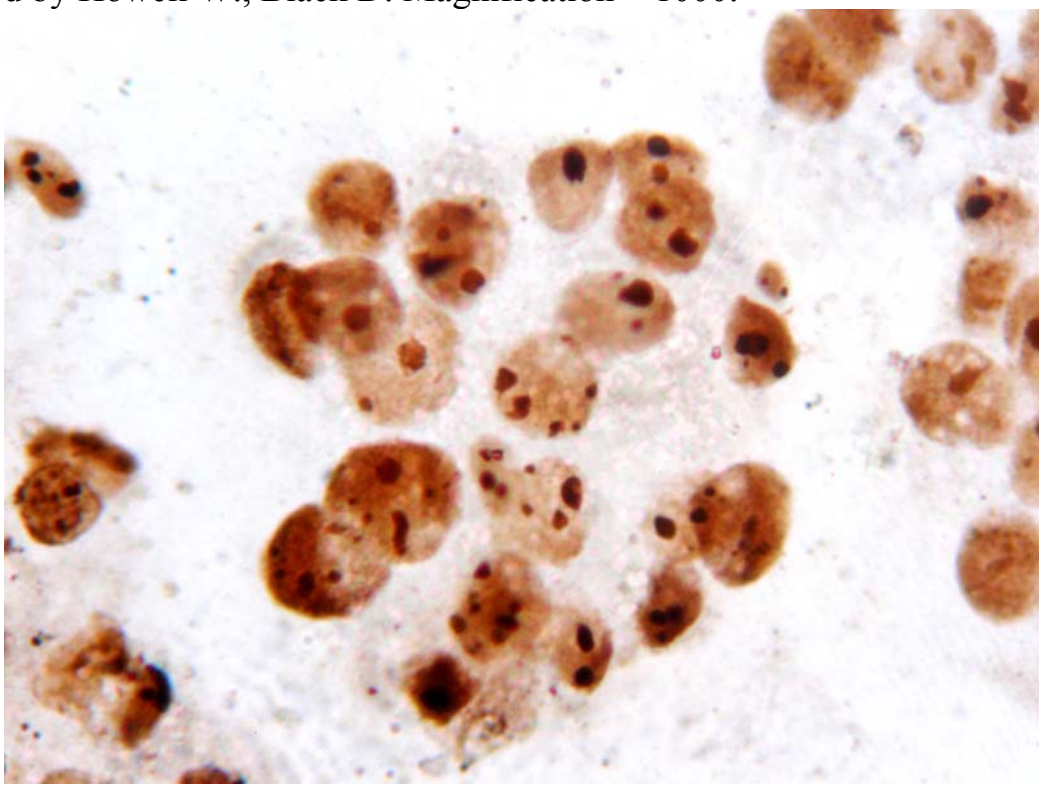

Fig. 7. The basic morphological types of nucleoli, predominantly transitional nucleolonemal forms, in the cells of moderately differentiated endometrioid carcinoma of the ovary. Stained by Howell W., Black D. Magnification ×1000.

Therefore, total count of morphofunctional types of nucleoli varied from 580 (5.80 ± 0.04) to 973 (9.73 ± 0.10), M = (7.95 ± 0.07). Typing showed that the average count and percentage of compact nucleoli ranged from (0.30 ± 0.01) 4.82% to (0.54 ± 0.02) 5.90%, M = (0.43 ± 0.01) 5.30%. Nucleolonemal nucleoli count in the nucleus varied from (1.49 ± 0.03) to (1.57 ± 0.02), percentage ranged from 15.50 to 25.70, when M = (1.52 ± 0.02) (19.80%). Underactive ring-shaped nucleoli count ranged from (0.89 ± 0.01) 15.36% to (1.96 ± 0.03) 20.66%, M = (1.53 ± 0.02) 19.0%. Inactive micronucleoli count was from (3.12 ± 0.04) 53.76% to (5.72 ± 0.07) 58.80%, M = (4.47 ± 0.05) 55.80%.

In all cases immunocytochemical study revealed positive expression with Ber-EP4 and CK 7 (Fig. 8) and negative expression with CK 20, which is an additional biomolecular sign of ovarian endometrioid carcinoma.

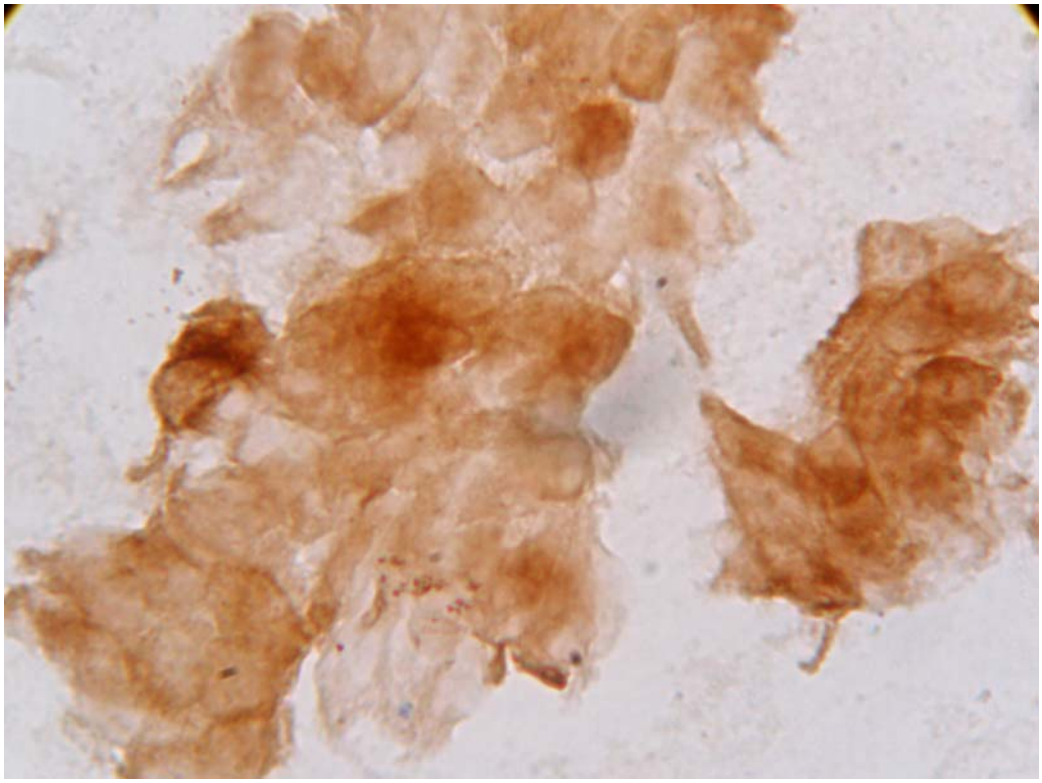

Fig. 8. Positive expression of mAb CK 7 in the OEC cells.

Conclusion

The study revealed certain cytomorphological, cytogenetical and immunocytochemical characteristics of ovarian endometrioid carcinoma (OEC). Thus, minimal signs of cell atypia are characteristic of this tumor histology type,

which makes it difficult to assess and causes low diagnostic efficacy of cytological diagnosis.

Results of the study of basic nucleoli types in OEC cells showed high counts in both total nucleoli and their active forms (compact and transitional nucleolonemal), which may be indicative of a poor prognosis of the disease.

Immunocytochemical study with mAb - CK7, CK20 and epithelial antigen (Ber-EP4) can be used as an additional method in the differential diagnosis of OC morphological variants.

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