FEATURES OF HORMONAL RECEPTOR STATUS,EXPRESSION OF VEGF,HER 2/neu IN SEROUS CANCER AND FUNCTIONAL CYSTSOF OVARIES

HORMONAL HOMEOSTASIS IN PATIENTS WITH RECURRENTSEROUS OVARIAN CANCER

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Hormonal homeostasis in patients with recurrent serous ovarian cancer and surgery menopause patients operated on the benign uterus pathology were studied in comparison; hormonal receptor status, expression of HER-2/neu and VEGF in cells of serous cancer and functional cysts of ovaries have been studied and compared.

Key words: *hormonal homeostasis, serous ovarian cancer, functional ovarian cysts, receptors to estrogens, progesterone, testosterone, expression of VEGF and HER-2/neu.*

The main reason of ineffective treatment of patients with ovarian cancer (OC) consists in its late diagnosis due to the lack of pathognomic symptoms. The maindifficulty consists in the fact that to date there is no final concept of OC pathogenesis, understanding of which would solve the problem of early diagnostics and carrying out the most effective pathogenetic treatment. However, for today significant amount of experimental, epidemiological and clinical facts have been accumulated, which have showed that the leading role in general hypothesis of the OC pathogenesis is played by interaction between complicated mechanisms of endocrine-metabolic and molecular-genetic disorders [1–5]. To the risk factors of this disease are referred ceaseless ovulation in consequence of shortening of number of pregnancies and childbirths in women, sterility of different genesis, using of ovulation-stimulating drugs, and, according to the results of recent studies – obesity, especially in women of perimenopausal period [6–9]. OC is characterized by pronounced heterogeneity of molecular-biological markers of tumor cells and their complicated interactions with factors of microenvironment that altogether forms tumor phenotype with significant variability [10–13]. Hormone-dependence of OC to date remains not fully studied. It is showed by the results of numerous studies, which have determined that ovaries are not only producing sex steroid hormones, but also acting as a target tissue for gonadotropic, classical and non-classical steroid hormones, as well as for gonadotropin-releasing hormone [14, 15]. However, data on clinical, prognostic and therapeutic significance of hormonal receptor status of OC are controversial. Moreover, no full presentation of mechanisms of realization of hormonal-receptor signal in ovarian tumor cells, whichinitiate processes of proliferation, invasion and metastasis, has been obtained. Results of our previous studies have demonstrated high frequency of positive expression of all steroid hormones

receptors (SHR) in OC cells that came as unfavorable factor of tumor process [16]. In contrast, according to the data of other studies, in benign cysts and morphologically unchanged ovaries, low frequency or lack of SHR expression has been determined [17]. However, there are also controversial scientific data [18–19]. Epidermal growth factor receptor 2 (EGFR-2 – HER-2/neu) and vascular endothelial growth factor (VEGF) play special role in mechanisms of auto- and paracrine regulation of reproductive system and, first of all, of ovarian function. Angiogenesis in menstrual cycle is controlled by many growth factors including HER-2/neu [20, 21]. Many authors have noted that high expression of HER-2/neu and VEGF in ovarian tumor cells are factors of aggressive clinical course and unfavorable prognosis of disease [22–25]. Moreover, it has been proved to date that estradiol induces VEGF expression and its receptors via estrogen-receptor signal pathways in cells of organs of reproductive system at physiological and tumor angiogenesis [26].

Some authors have observed disorder of hormonal homeostasis in OC patients – hyperestrogenemia and hyperandrogenemiaon the background of absolute hypoprogesteronemia – as compared with ones in almost healthy women of the same age period [27]. However, there are also controversial scientific data [28, 29].

The question of hormonal therapy use in mentioned category of patients still remains discussible. Over many years, hormonal treatment has been prescribed empirically as “therapy of despair” for patients with chemoresistant and relapsing OC, when rest of therapeutic methods sputtered out having low index of effectiveness [3, 14].

Since till now no full presentation of disorders of hormonal homeostasis in patients with relapsing OC and significant differences between hormonal receptor status, expression of HER-2/neu, VEGF in cells of serous OC and ovaries at benign hormone-dependent pathology of reproductive system has been obtained, solution of these questions will allow to specify pathogenesis of OC and will have significance for substantiation of indication to hormonal therapy as component of complex treatment of patients.

AIM OF THE STUDY was to investigate and compare hormonal homeostasis of patients with relapsing OC (studied group) and patients with surgical menopause, who underwent surgery on account of benign pathology of uterus (control group); to study and compare hormonal receptor status, expression of HER-2/neu and VEGF in cells of serous OC and ovaries of patients from control group.

MATERIALS AND METHODS

Patients.41 patients with relapsing serous OC of stage III, who underwent combined treatment within 2011–2012 (in accordance with the standards of diagnostics and treatment of cancer patients approved by the Order of MoH of Ukraine № 554 from 17.09.2007) in National Cancer Institute, Kyiv Clinical Hospital and Dnepropetrovsk Clinical Regional Dispensary, have been involved in the study. Combined treatment included cytoreductive surgery with following chemotherapy in adjuvant regimen or

in combination with neoadjuvant chemotherapy. All patients underwent chemotherapy with combination of platinum drugs and taxane-containing or platinum with cyclophosphamide. Relapse of disease has been determined 12–18 months after the end of combined treatment. Moreover, 35 women with surgical menopause, who underwent surgical treatment in the volume of extirpation of uterus with appendages on account of leiomyoma of uterus in Central City Hospital of Novomoskovsk of Dnepropetrovsk Region within 2011–2012 (control group), have been included in the study.

Diagnosis of serous OC and benign cyst tumor-like masses of ovary has been verified using morphological study of surgical material of patients of two groups according to the WHO histological classification of ovarian tumors (2002), clinical standardizing of OC – in accordance with the international FIGO classification (2009). Diagnosis of leiomyoma of uterus has been verified using morphological study of surgical material of patients from the control group according to the WHO histological classification of mesenchymal tumors of body of uterus (2003). All patients have given their informed consent for using surgical material in diagnostic purposes.

Results of the studies have been evaluated depending on the age periods of women of two groups according to the WHO classification (1980): reproductive (up to 45), perimenopause (46-55), early postmenopause (56–60) and late postmenopause (61 and up). Since small sampling of patients took part in the study, postmenopausal women were not divided into the patients of early and late postmenopause.

Results of our previous studies have showed that neoadjuvant chemotherapy does not influence the expression of SHR and there is no correlation between the latter and grade of differentiation of ovarian tumors of different histogenesis. For this reason, we have not distributed OC patients depending on adjuvant or neoadjuvant chemotherapy and differentiation of serous OC [16].

Immunohistochemical analysis

Levels of expression SHR, VEGF and HER-2/neu in ovarian tumor cells of patients with OC relapse (OCR) and ovaries of patients of control group have been evaluated in age period at the moment of surgical treatment. Immunohistochemical study of receptors of estrogens (ER), progesterone (PR), testosterone (TR), VEGF and HER-2/neu in cells of ovary has been carried out on paraffin sections 4-5 micron thickness placed on glasses processed with poly-L-lysine. As primary anibodies, monoclonal antibodies specific to ER have been used(anti-Human Estrogen Receptor alfa Clone 1D5, DakoCytomation, Denmark), PR (anti-Human Progesterone Receptor Clone PgR 636, DakoCytomation, Denmark), TR (testosterone antibody Clone GTX72779, GeneTex, USA),VEGF (anti-VEGF RB-9031-P1, Thermo Scientific, USA)and HER-2/neu (anti-c-erbB2 Clone SP3, Thermo Scientific, USA).Forvisualizationoftheresultsofreaction, EnVisionsystem (DakoLSAB2system, Denmark)reagentskithasbeenusedin accordance withthemanufacturer recommendations, sectionswerestainedwithMayer hematoxylin. For evaluation of immunohistochemical expression of ER, PR, TR, VEGF and HER-2/neu, semi-quantitative method has been applied. When specific nuclear staining for steroid receptors, membrane-cytoplasm staining for VEGF and HER-2/neu were present,

number of immune-positive and immune-negative cells in percentage has been determined. In each histological specimen, expression of steroid receptors, VEGF and HER-2/neu in 1000 cells has been analyzed. For total evaluation of SHR, VEGF and HER-2/neu expression in ovarian cells, methods applied by us earlier have been used [16, 25, 30]. Level of expression of SHR was expressed in scores: 0 score – lack of staining of nuclei of ovarian cells; 1 score – poor staining of nuclei of ovarian cells – ≤ 10 %of cells; 2 scores – moderate staining – 11–50 %; 3 scores – high staining – 51–80 %; 4 scores – hyperexpression – >80 % of stained ovarian cells. Number of ovarian cell nuclei with more than 10% of moderate and high grade of staining was taken as a positive expression of steroid receptors. Level of VEGF expression has been also evaluated in scores: 0 score – lack of staining of cytoplasm and membrane of ovarian cells; 1 score – poor staining – 1-25% of ovarian cells (VEGF+); 2 scores – moderate level of proportional membrane-cytoplasm staining – 26%-50% of cells (VEGF++); 3 scores – high level of staining or hyperexpression – more than 50% of ovarian cells (VEGF+++). Over 25% of cells with moderate and high expression was taken as positive VEGF expression (VEGF+). Level of expression of receptor HER-2/neu has been evaluated in scores: 0/1+ - lack or poor staining of cytoplasm and membrane of ovarian cells, 2+ - moderate level of proportional staining of cytoplasm and membrane of ovarian cells, 3+ - high level and 4+ - hyperexpression of ovarian cells with intensively stained cytoplasm and membrane. Over 10% of cells with moderate and high membrane-cytoplasm staining was taken as positive expression of HER-2/neu. Monoclonal antibodies against pan-cytokines were used as positive control. Buffered physiological solution, which was put on histological section instead of monoclonal bodies, was used as a negative control.

Immunochemical analysis of hormonal homeostasis of OCR patients and patients of control group has been carried out in the laboratory "Synevo". Level of serum hormones follicle-stimulating (FSH), luteinizing(LH), estradiol (E_2), progesterone and testosterone (free) in patients of two groups was evaluated in age period at the moment of carrying out this study. Analysis of serum hormones in OCR patients was carried out when relapse of disease was diagnosed (12–18 months after the end of primary treatment) beforecarrying out drug therapy, in women of control group – 12–18 months after surgical treatment. Patients of two groups have given informed consent for study of their hormonal homeostasis.

Levels of FSH, LH, E_2 and progesteronein blood serum were determined by immunohistochemical method with electrochemiluminescencedetection (ECLIA) using test-system RocheDiagnostics (Switzerland), analyzer Cobas 6000 and reagents kit: "ElecsysFSH" (1775863), "ElecsysLH" (1732234), "Elecsys Estradiol II" (03000079), "Elecsys Progesterone II" (2145383). Principle of method (principle of "sandwich") lied in formation of specific immune complex marked with ruthenium generating electrochemical luminescence. Level of testosterone (free) was determined by solid-phaseimmune-enzyme method using test-system and analyzer EUROIMMUN (Germany), and reagents kit "Free Testosterone ELISA" (AA E-1400). For positive and negative probe, finished control sera with adjusted parameters, which were provided by each manufacturer in each test-system, have been

applied. Results have been evaluated according to the range of measurements given in each test-system. Concentration of each hormone in samples (blood serum of patients) has been calculated automatically in the following units: for FSH and LH in mIU/ml, E_2 and free testosterone in pg/ml,progesterone –ng/ml.

Statistical analysis of obtained data was carried out using non-parametric and parametric statistics including Student's *t*-criterion. Significance of intergroup differences has been evaluated by *U*-criterion of Mann-Whitney. Statistically significant were considered data at $p<0.05$. Processing of the results of study has been carried out using program software STATISTICA 6.0.

RESULTS AND DISCUSSION

Age of OC patients at the moment of surgical treatment varied from 34 to 68 being in average 50.3±1.3, patients of control group – 47.1±1.3 (38–62). Differences by age of patients of two groups statistically were insignificant ($p=0.25$).

Analysis of morphological structure of ovary in women of control group is represented in Table 1. Data of this Table show that most of patients had functional cysts of ovary (17/48.6 %): follicular (4/11.4 %) and cysts of corpus luteum (13/37.2 %). Only in 8.6% of women of control group, serous cysts of ovaries without epithelial lining, which are known to be final stage of development of some follicular cysts and cysts of corpus luteum, have been detected. Loss of epithelial lining resulting from exfoliating or atrophy, and frequent sclerosing of underlying layers do not give opportunity to specify histogenesis of these cysts at morphological study [31]. Functional cysts of ovary originate from physiological structures. Persistence of non-ovulated follicle, in cavity of which follicular liquid is accumulated, leads to the formation of follicular cysts. Walls of cyst contain granulose and teca-cells. Follicular cysts are result of multifactor causes, leading role among which is given to disorder in system of hypothalamus-hypophysis-ovaries that causes the desynchronizing of activity of biochemical factors participating in intra-follicular mechanism of ovulation including prostaglandins, proteolytic enzymes, oxytocin and relaxin [20, 32]. Formation of cysts of corpus luteum is also caused by hormonal misbalance and disorder of blood circulation and lymph drainage in ovarian tissues [21]. Luteinized granulose and teca-cells are located in wall of cyst in the form of layer or particular groups. Normally corpus luteum has the highest level of vascularization in human organism. This process is directly regulated by high VEGF expression. Angiogenic activity is located under the control of many growth factors including HER-2/neu [20, 21].

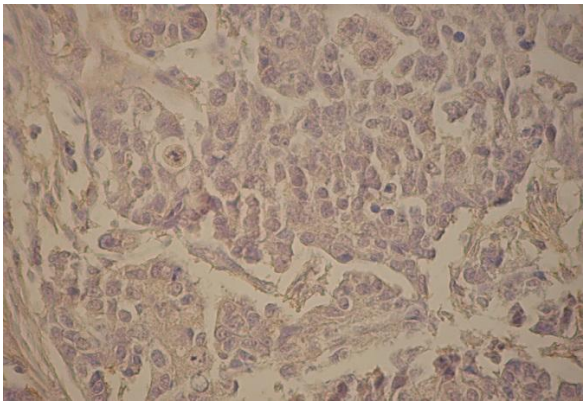
Results of immunohistochemical tests of expression of SHR, HER-2/neu and VEGF in serous OC show high frequency of positive expression of ER, PR, TR, HER-2/neu and VEGF in cells of serous OC (65.9 %, 63.4 %, 56.1 %, 65.9 and 48.8 %, respectively) (Table 1, Fig. 1–2). When analyzing results of expression of SHR, HER-2/neu and VEGF in ovaries of patients of control group, total lack of expression of listed markers in epithelial, stromal,granulose and teca-cells of morphologically unchanged ovary, ovary with involutive changes, as well as ovary with serous cysts, has been determined. However, high frequency of positive expression of SHR, HER-2/neu and VEG in granulose, teca- and lutein cells of functional cysts of ovary has been observed: follicular – 75.0 %, 50.0 %, 25.0 %, 75.0 and 50.0 %,

respectively, corpus luteum cysts – 65.1 %, 76.9 %, 65.1 %, 92.3 and 69.2 %, respectively. Differences in frequency of expression of SHR, HER-2/neu, VEGF in serous OC and functional cysts had no significance ($p>0.05$). Exception was represented by significantly lower frequency of expression of TR in cells of cysts of corpus luteum as compared with one in follicular cysts and serous OC ($p=0.03$). As we have previously determined, pronounced expression of TR in serous OC is a factor of unfavorable clinical course of tumor process [16, 30].

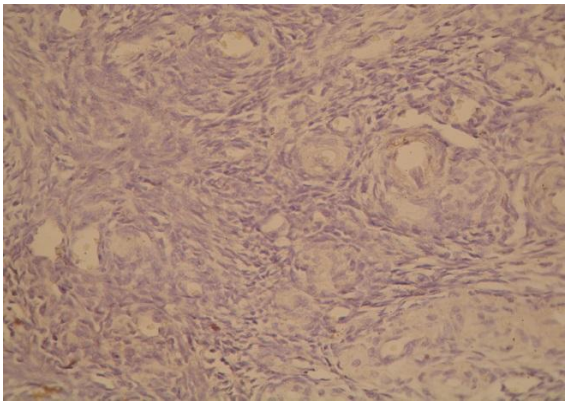
Table 1

Expression of SHR, HER-2/neu, VEGF in cells of serous OC in patients of studied group and ovaries in patients of control group

Serous OC, **n=41/100%**	**Patients of studied group (n=41)**									
	ER +		**PR +**		**TR +**		**HER-2/neu**		**VEGF**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
	27	**65.9**	**26**	**63.4**	**23**	**56.1**	**27**	**65.9**	**20**	**48.8**
Morphological structure of ovaries	**Patients of control group (n=35)**									
	ER +		**PR +**		**TR +**		**HER-2/neu**		**VEGF**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Normalstructureofovary,**n=4/11.4%/100%**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Involutivechanges, **n=11/31.4%/100%**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Serous cysts, **n=3/8.6%/100%**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Follicular cysts, **n=4/11.4%/100%**	**3**	**75.0**	**2**	**50.0**	**1**	**25.0**	**3**	**75.0**	**2**	**50.0**
Corpus luteum cysts, **n=13/37.2%/100%**	**8**	**61.5**	**10**	**76.9**	**8**	**61.5**	**12**	**92.3**	**9**	**69.2**



A



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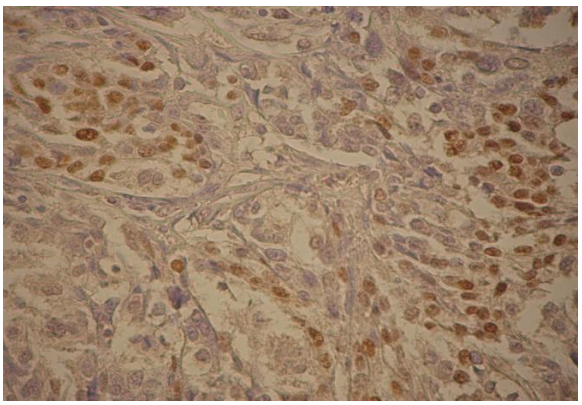

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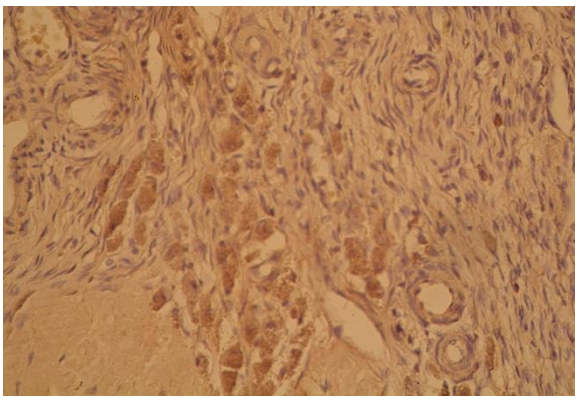

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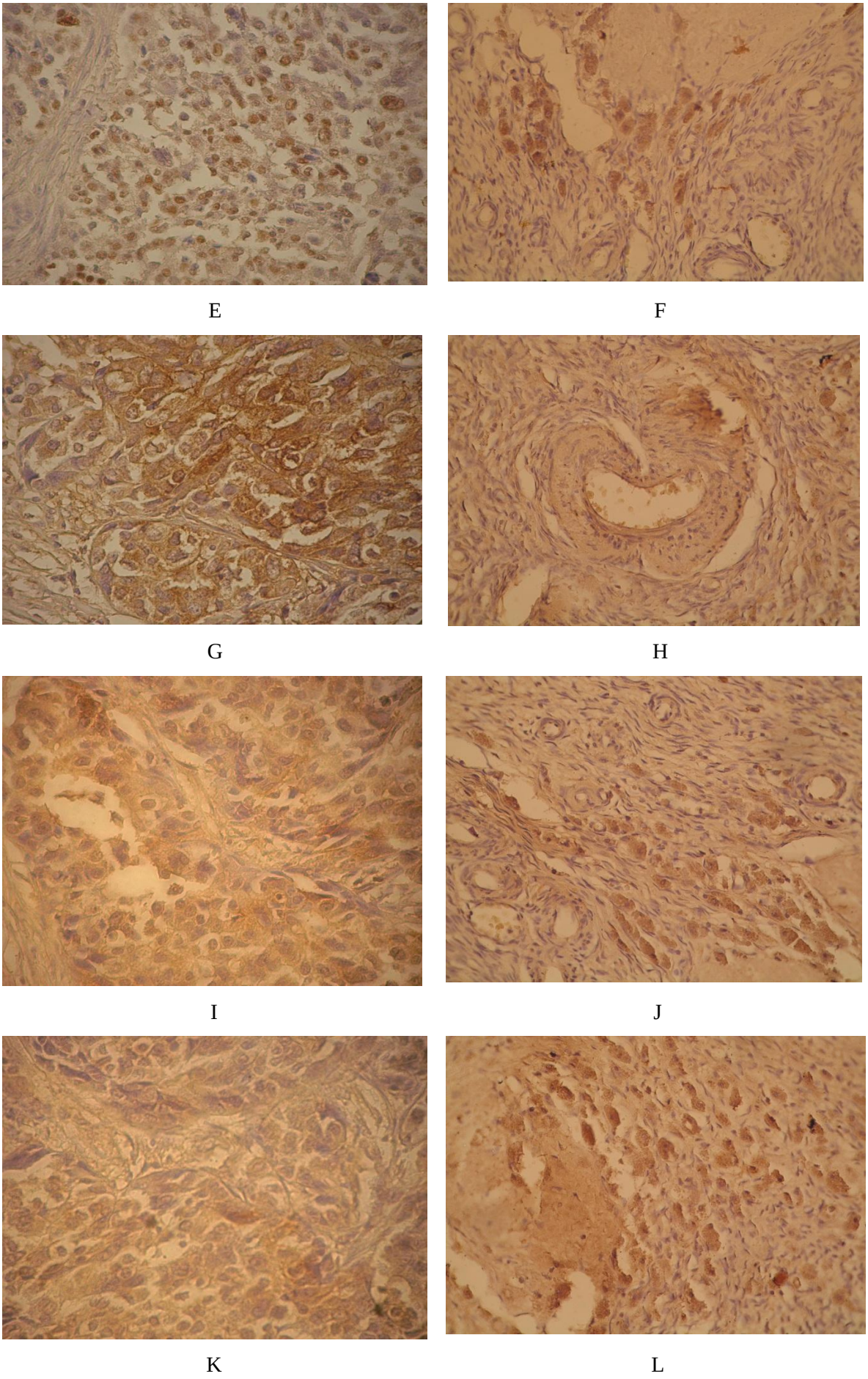

E F G H I J K L

Fig.1. Immunohistochemical picture in cells of serous OC and cysts of corpus luteum, ×400:

A – lack of expression of studied markers in cells of serous OC;

B – lack of expression of studied markers in cells of cysts of corpus luteum;

C – high expression of ER in cells of serous OC;

D – moderate expression of ER in cells of cyst of corpus luteum;

E – high expression of PR in cells of serous OC;

F – moderate expression of PR in cells of cyst of corpus luteum;

G – high expression of TR in cells of serous OC;

H– moderate expression of TR in cells of cyst of corpus luteum;

I – high expression of HER-2/neu in cells of serous OC;

J – high expression of HER-2/neu in cells of cyst of corpus luteum;

K – high expression of VEGF in cells of serous OC;

L – high expression of VEGF in cells of cyst of corpus luteum.

Thus, high frequency of expression of SHR, HER-2/neu, VEGF in cells of serous OC and functional cysts of ovary allow to assume presence of common risk factors and mechanisms of development of OC and benign hormone-dependent diseases of uterus and ovary.

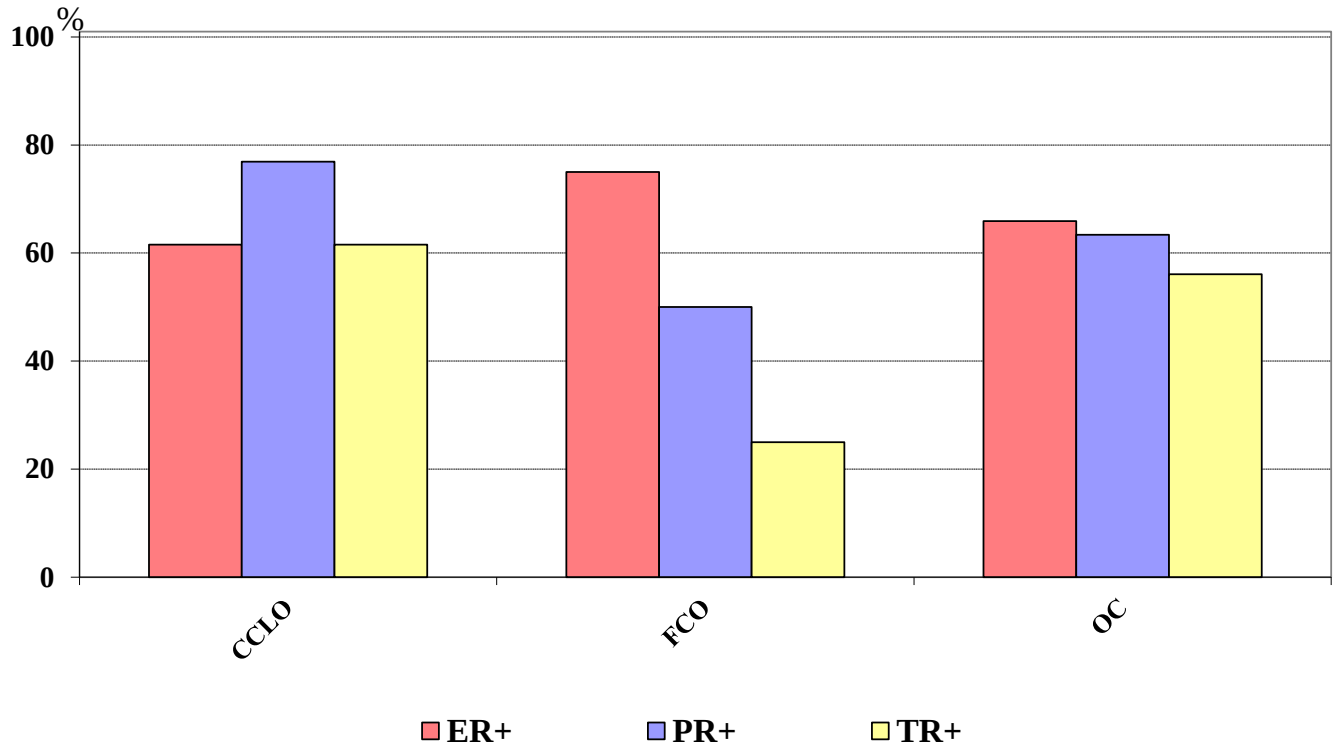

Fig. 2.Frequency of expression of SHR in cells of serous OC and functional cysts of ovary

Note:

CCLO – cyst of corpus luteum of ovary;

FCO– follicular cyst of ovary;

OC – ovarian cancer.

Further we have analyzed and compared frequency of expression of SHR, HER-2/neu, VEGF in serous OC and functional cysts of ovary of patients among the studied and control groups depending on age period (Table 2, Fig. 3–7). As data from Table 2 and Fig. 3–7 show, the highest number of estrogen-, progesterone- and testosterone-receptor-positive tumors was observed in patients with OCR of perimenopausal age – 63.0 %, 61.5 and 65.2 %, respectively. At the same time, the highest number of VEGF and HER-2/neu-positive tumors also was detected in patients of this age period (60.0 and 55.6%, respectively). However, it should be mentioned that the most part of patients with OCR have constituted women of perimenopausal age (56.1%). Second place by frequency of estrogen-, progesterone-, testosterone-receptor-positive tumors is occupied by patients of postmenopausal period (22.2%, 26.9 and 21.7%, respectively). The lowest frequency of receptor-positive serous OC was observed in patients of reproductive age: by ER – 14.8 %, PR – 11.6 % andTR – 13.1 %. Differences between rates of frequency

of expression of SHR in serous OC of patients of different age periods are reliably significant (reproductive period and perimenopause – p=0.01, perimenopause and postmenopause– p=0.03, postmenopause and reproductive period – p=0.04). Results of our previous studies and studies of the other authors have showed that OC patients in perimenopausal and postmenopause periods have the most aggressive clinical course and unfavorable prognosis of disease. Moreover, the highest frequency of tumors with positive hormonal-receptor status was detected in patients with serous OC of postmenopausal age. Survival of these patients was significantly lower, than of patients with receptor-negative serous OC [1, 3, 4, 16].

Table 2

Frequency of expression of SHR, HER-2/neu, VEGF in cells of serous OC of patients with OCR and functional cysts of ovary in patients of control group depending on age period

Age period, n=41/100%	**Patientswithreceptor-positiveserous OC (studied group, n=41)**									
	ER +		**PR +**		**TR +**		**HER-2/neu**		**VEGF**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Reproductive period, n=8/19.5%	**4**	**14.8**	**3**	**11.6**	**3**	**13.1**	**7**	**25.9**	**4**	**20.0**
Perimenopause, n=23/56.1%	**17**	**63.0**	**16**	**61.5**	**15**	**65.2**	**15**	**55.6**	**12**	**60.0**
Postmenopause, n=10/24.4%	**6**	**22.2**	**7**	**26.9**	**5**	**21.7**	**5**	**18.5**	**4**	**20.0**
Total	**27**	**65.9/ 100**	**26**	**63.4/ 100**	**23**	**56.1/ 100**	**27**	**65.9/ 100**	**20**	**48.8/ 100**
Age period, n=35/100%	**Patientswithreceptor-positivefunctionalovariancysts (control group, n=35)**									
	ER +		**PR +**		**TR +**		**HER-2/neu**		**VEGF**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Reproductive period, n=18/51.4%	**11**	**100.0**	**11**	**91.7**	**8**	**88.9**	**13**	**86.7**	**10**	**90.9**
Perimenopause, n=12/34.3%	**0**	**0.0**	**1**	**8.3**	**1**	**11.1**	**2**	**13.3**	**1**	**9.1**
Postmenopause, n=5/14.3%	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Total	**11**	**31.4/ 100**	**12**	**34.3/ 100**	**9**	**25.7/ 100**	**15**	**42.9/ 100**	**11**	**31.4/ 100**

Analysis of dependence of expression of SHR in cells of serous OC between each other (Table 3) has showed direct correlation between frequency of expression of ER and PR (p=0.001), ER and TR (p=0.003), PR and TR (p=0.002), especially significant between ER and PR (r=0.823). Moreover, significant correlation between frequency of expression of ER, PR and age period of patients with OCR (r=0.354, p=0.001 и r=0.342, p=0.031, respectively), and lack of correlation for TR (r=-0.117, p=0.6) has been detected. Obtained data coincide with the results of studies previously conducted by us [16].

Frequency of VEGF expression in tumor ovarian cells of patients with relapsing OC of reproductive and postmenopausal age was the same – 20.0% (Table 2). Differences in rates of frequency of expression of HER-2/neu in serous OC of patients of these age periods (25.9 and 18.5%, respectively) had no significance ($p>0.05$). Moreover, statistical analysis has showed lack of correlation between frequency of expression of HER-2/neu, VEGF in serous OC and age of patients ($r=-0.014$, $p=0.85$ and $r=-0.036$, $p=0.73$, respectively). At the same time, significant correlation of expression of HER-2/neu with expression of VEGF in cells of serous OC has been determined ($r=0.925$, $p=0.001$) (Table 3). Thus, represented data confirm obtained results of previously conducted by us studies [25, 30].

Evaluation of correlation between expression of HER-2/neu, VEGF and expression of SHR in ovarian tumor has showed lack of significant correlation between them (Table 3). Obtained data also fit the results of previously conducted studies, which have determined that expression of HER-2/neu and VEGF in serous OC are independent factors of aggressive and unfavorable course of tumor process [25, 30].

Analysis of frequency of expression of ER, PR, TR, HER-2/neu and VEGF in cells of functional ovarian cysts of patients from control group has determined the highest rates among women of reproductive period –100 %, 91.7 %, 88.9%, 86.7 and 90.9 %, respectively. Only in single cases, expression of these markers was observed in functional ovarian cysts of women of perimenopausal age (Table 2 and Fig. 2–6). Obtained data can be explained by age physiological condition of patients of control group, since presence of functional ovarian cysts is typical only for menstruating women [31, 32].

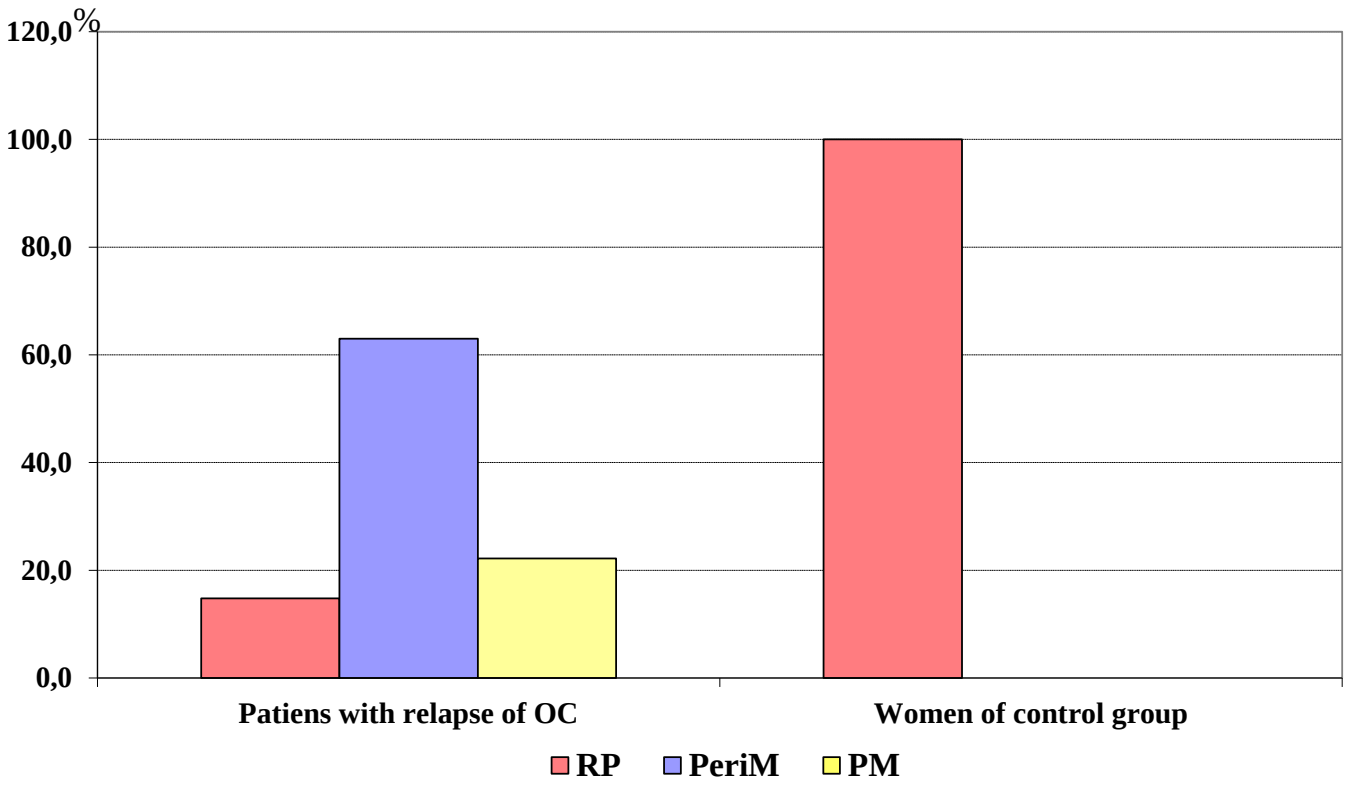

Fig. 3.Frequency of ER expression in cells of serous OC and functional ovarian cysts in patients of the studied and control groups depending on age period

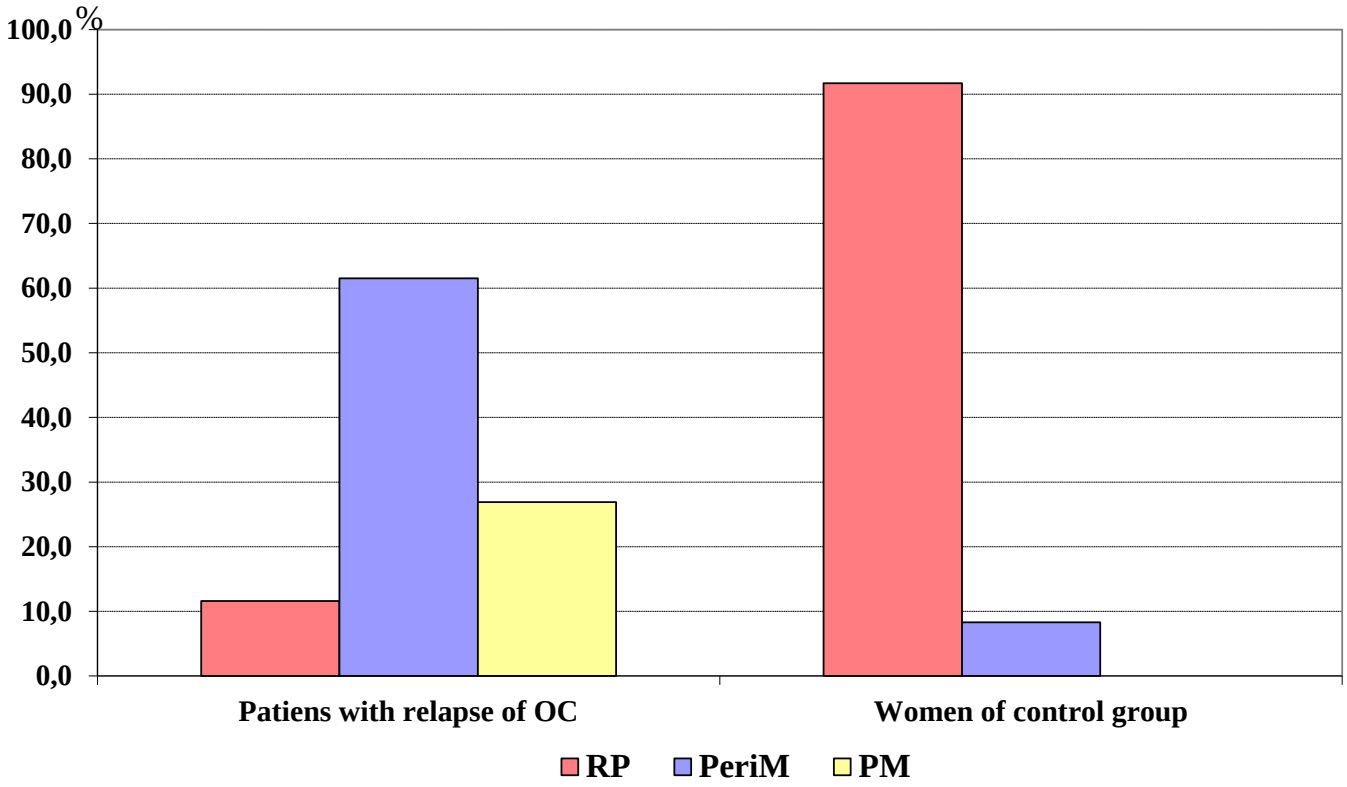

Fig. 4.Frequency of PR expression in cells of serous OC and functional ovarian cysts in patients of the studied and control groups depending on age period

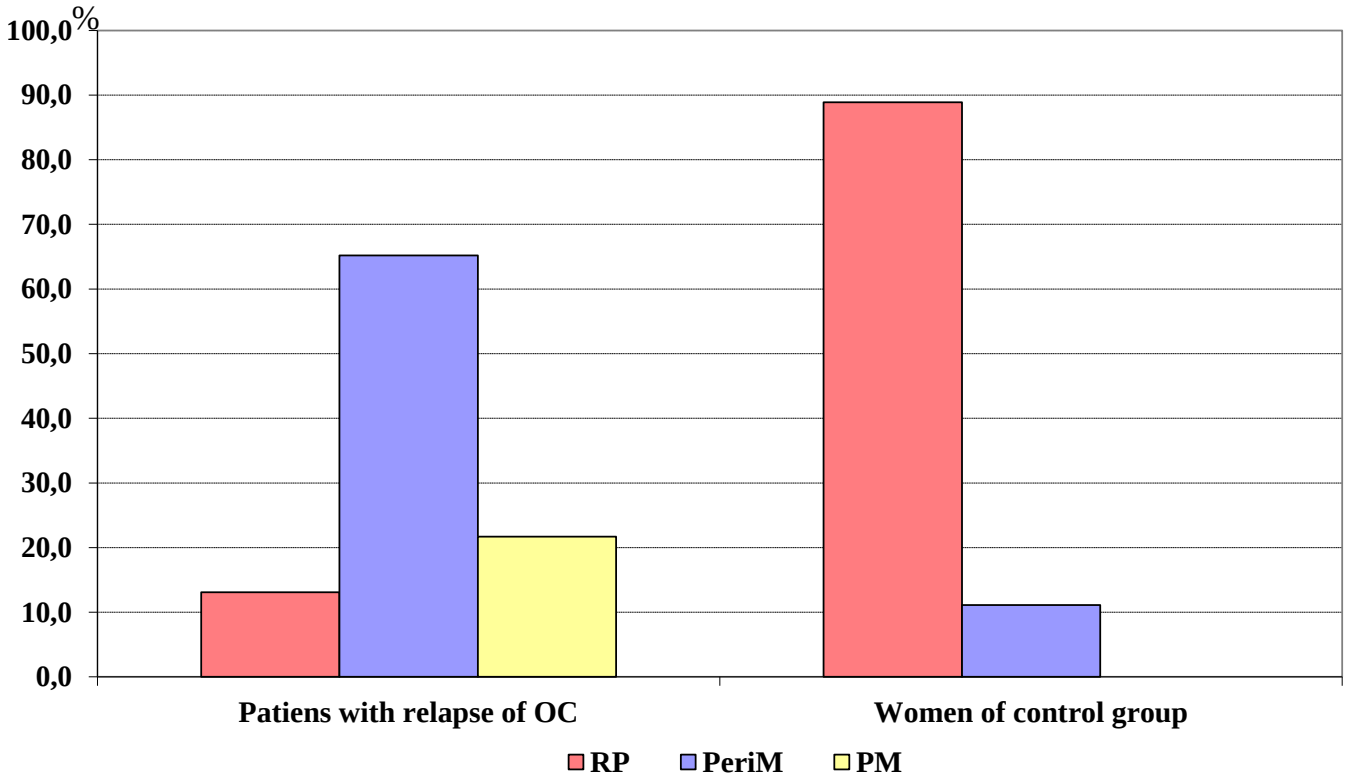

Fig. 5.Frequency of TR expression in cells of serous OC and functional ovarian cysts in patients of the studied and control groups depending on age period

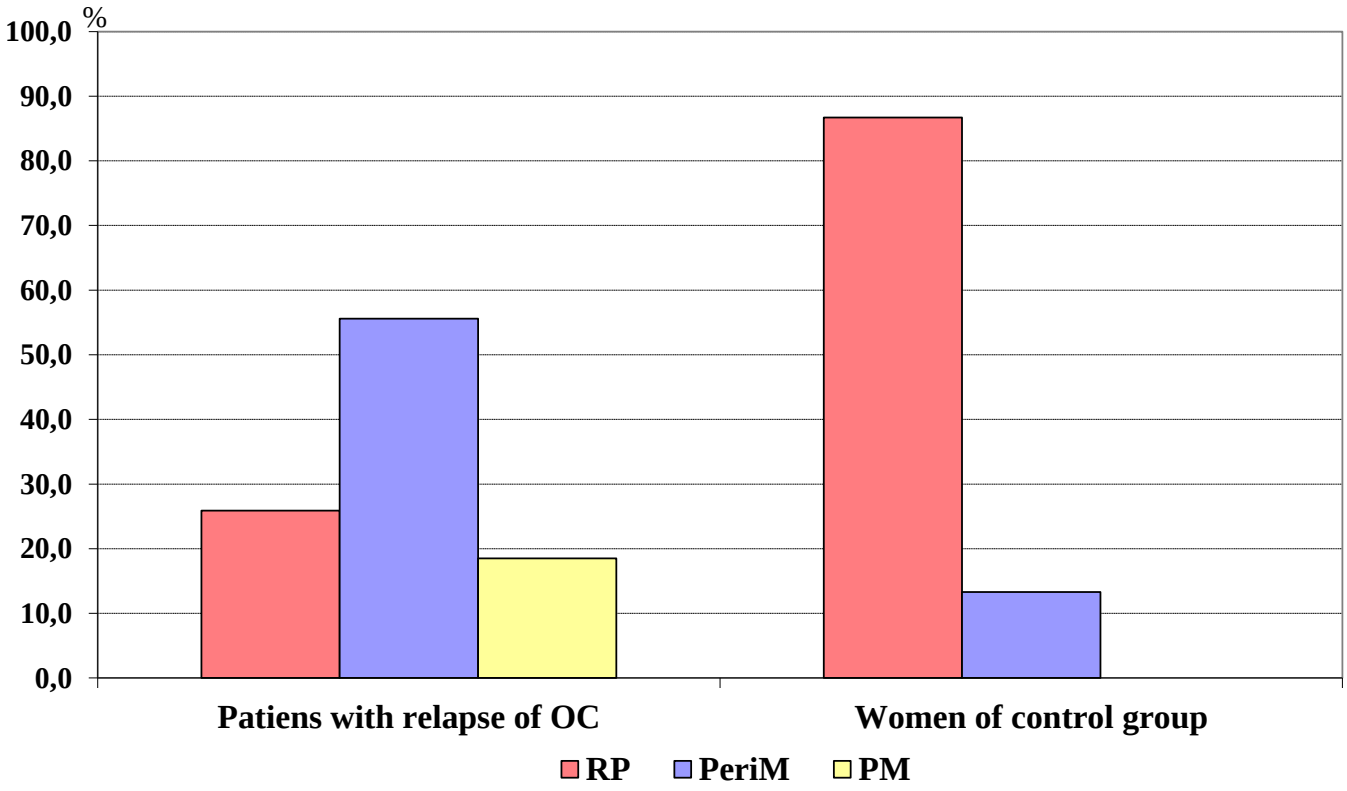

Fig. 6.Frequency of HER-2/neu expression in cells of serous OC and functional ovarian cysts in patients of the studied and control groups depending on age period

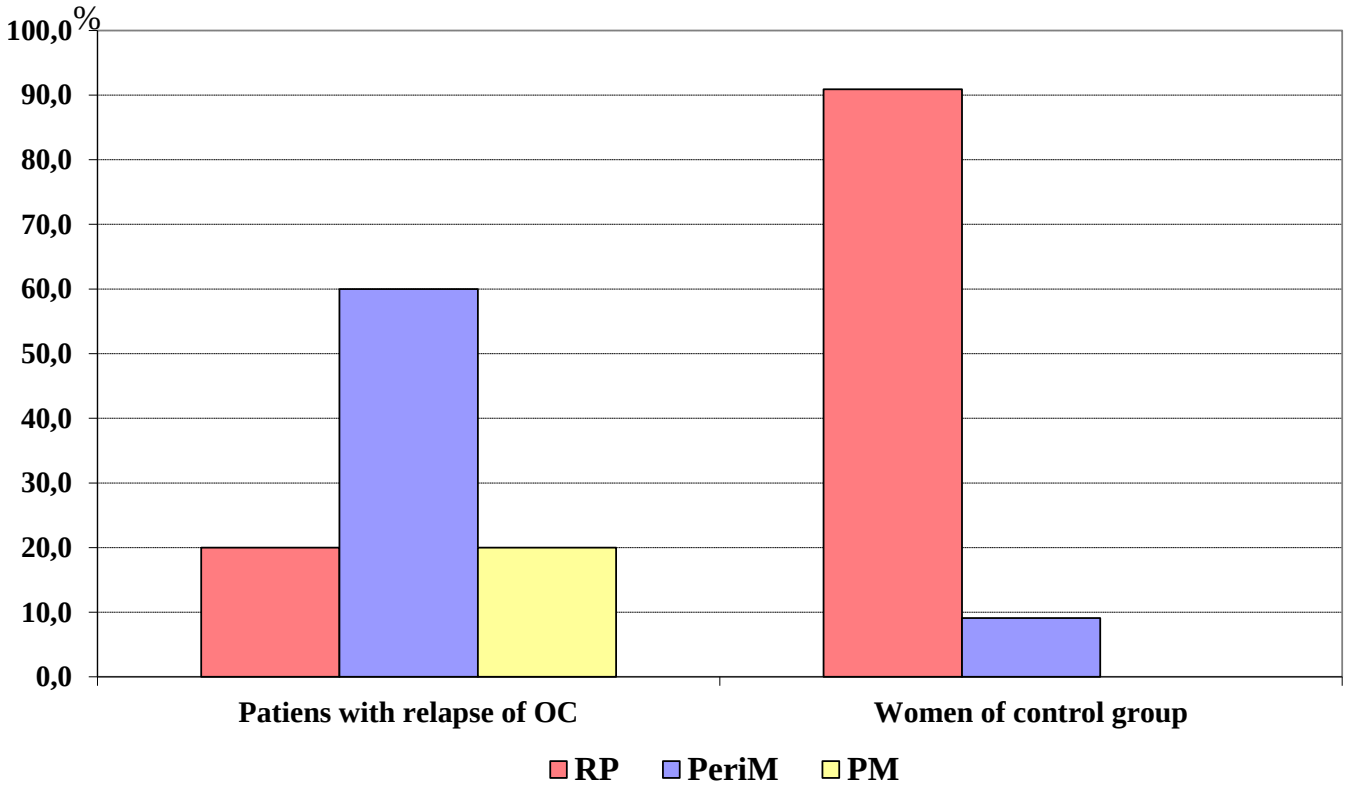

Fig. 7.Frequency of VEGF expression in cells of serous OC and functional ovariancysts in patients of the studied and control groups depending on age period

Note:
RP – reproductive period;
PeriM – perimenopause;
PM – postmenopause.

Later on we have evaluated rank correlations between expression of SHR, HER-2/neu, VEGF in functional ovarian cysts and age of patients of control group (Table 4). As data from Table 4 show, significant correlations between all rates have been determined. Exception is lack of correlation between expression of TR and VEGF (r=0.173, p=0.46), expression of HER-2/neu, VEGF and age period of women (r=-0.073, p=0.7 and r=-0.026, p=0.91, respectively). Obtained data confirm direct participation of expression of SHR, HER-2/neu and VEGF in granulose, teca- and lutein cells of ovary in regulation of normal ovarian-menstrual cycle in women [20, 21, 32].

Table 3

Rank correlation of Gamma significant on the levelp<0.05, between expression of SHR, HER-2/neu,VEGF, age period of patients with relapsing serous OC (n=41)

Rate	Correlation coefficient	p
ER& age period	**0.354**	**0.001**
ER&PR	**0.823**	**0.001**
ER&TR	**0.426**	**0.003**
ER &VEGF	0.254	0.151
ER &HER-2/neu	-0.112	0.462
PR&ageperiod	**0.342**	**0.031**
PR&ER	**0.823**	**0.001**
PR&TR	**0.397**	**0.002**
PR& HER-2/neu	0.125	0.317
PR&VEGF	0.027	0.763
TR&ageperiod	-0.117	0.591
TR &ER	**0.426**	**0.003**
TR &PR	**0.397**	**0.002**
TR&HER-2/neu	0.012	0.879
TR&VEGF	0.059	0.692
HER-2/neu&age period	-0.014	0.851
HER-2/neu&ER	-0.112	0.462
HER-2/neu&PR	0.125	0.317
HER-2/neu&TR	0.012	0.879
HER-2/neu&VEGF	**0.925**	**0.001**
VEGF &age period	-0.036	0.727
VEGF &ER	0.254	0.151
VEGF &PR	0.027	0.763
VEGF &TR	0.059	0.692
VEGF& HER-2/neu	**0.925**	**0.001**

Table4

Rank correlation of Gama significant on the level p<0.05, between expression of SHR, HER-2/neu, VEGF, age period of patients with functional cysts of ovaries (n=35)

Rate	Correlation coefficient	p
ER&ageperiod	**-0.658**	**0.001**
ER&PR	**0.871**	**0.001**
ER&TR	**-0.726**	**0.001**
ER&VEGF	**0.554**	**0.005**
ER&HER-2/neu	**0.627**	**0.002**
PR&age period	**-0.497**	**0.032**
PR&ER	**0.871**	**0.001**
PR&TR	**-0.645**	**0.002**
PR& HER-2/neu	**-0.425**	**0.031**
PR&VEGF	**0.627**	**0.002**
TR&age period	**0.526**	**0.017**
TR &ER	**-0.526**	**0.016**
TR&PR	**-0.645**	**0.002**
TR &HER-2/neu	**0.523**	**0.012**
TR&VEGF	0.173	0.456
HER-2/neu&age period	-0.073	0.701
HER-2/neu&ER	**0.627**	**0.002**
HER-2/neu&PR	**-0.425**	**0.031**
HER-2/neu&TR	**0.523**	**0.012**
HER-2/neu&VEGF	**0.658**	**0.001**
VEGF &age period	-0.026	0.912
VEGF &ER	**0.554**	**0.005**
VEGF &PR	**0.627**	**0.002**
VEGF &TR	0.173	0.456
VEGF& HER-2/neu	**0.658**	**0.001**

We have carried out analysis of expression of SHR, HER-2/neu, VEGF in cells of serous OC and functional ovarian cysts, the results are represented in Table 5 and Fig. 8. Data in Table 5 show that there was significantly low expression of ER, PR and RT in functional ovarian cysts (10.0±3.3 %, 12.6±4.1 and 10.0±3.8 %, respectively). At that mean level of expression of ER and TR can be evaluated as negative. In contrast, in serous OC was determined moderate expression of SHR, the highest expression was observed for ER (33.7±4.9 %). Thus, mean level of expression of SHR in serous OC is significantly higher as compared with one in functional ovarian cysts (for ER – p=0.0002, PR – p=0.009, TR – p=0.02). Such tendency was observed also at evaluation of mean level of expression of HER-2/neu in OC cells and functional ovarian cysts (38.5±5.6 against 18.6±4.6 %, p=0.008). Mean level of

VEGFexpression in serous OC was also higher (28.9±4.7 %) as compared with one in functional ovarian cysts (16.9±4.7 %), but statistical analysis didnot detect significant differences between rates (p=0.08).

Table 5

Mean levels of expression of SHR, HER-2/neu, VEGF
in cells of serous OC and functional ovarian cysts

Rate, %	Serous OC, n=41	Functional ovarian cysts, n=35	p
ER	33.7±4.9	10.0±3.3	**0.0002**
PR	29.5±4.8	12.6±4.1	**0.009**
TR	23.7±4.1	10.0±3.8	**0.02**
HER-2/neu	38.5±5.6	18.6±4.6	**0.008**
VEGF	28.9±4.7	16.9±4.7	0.08

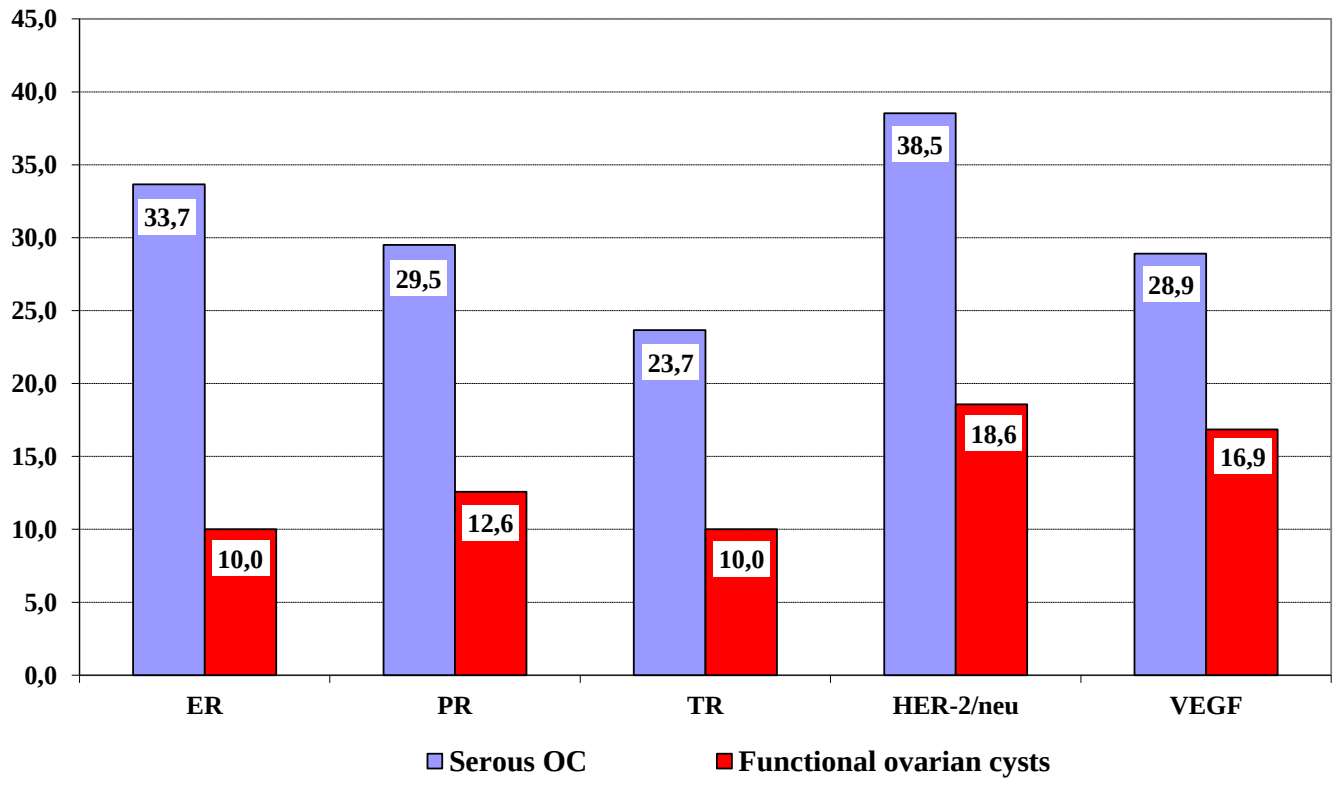

Fig. 8.Mean levels of expression of SHR, HER-2/neu andVEGF
in cells of serous OC and functional ovarian cysts

Later on study of hormonal homeostasis in patients with relapsing serous OC and patients of control group have been conducted, results are represented in Table 6. It should be mentioned that period of surgical menopause inpatients ofstudied and control groups was the same (≈ 18 months). As data in Table 6 show, mean age of patients with relapsing OC and patients of control group statistically was insignificant (51.1±1.3 and 48.7±1.3.respectively, p=0.25). When analyzing mean levels of gonadotropic and peripheral steroid sex hormones in blood serum of patients in studied and control groups, lack

ofsignificant differences in rates of hormonal homeostasis of patients of two groups was determined: for FSH – p=0.15, LH – p=0.23, E_2 – p=0.29, progesterone – p=0.07, testosterone – p=0.19. Rates of hormonal homeostasis of patients in these groups fit referential values of serum levels of sex hormones in postmenopausal period. It is confirmed by the fact that evaluation of levels of hormones in blood serum in women of postmenopausal period does not give full information on possible variants of development of pathological processes, since realization of hormonal effect needs presence of sufficient level of steroid receptors in cells of target tissue. Moreover, estrogenization in women of postmenopausal period is determined mostly not by the level of hormones in blood serum, but by the local concentration of them in tissues [2, 33].

Table 6

Mean levels of gonadotropic and peripheral steroid hormones in blood serum in patients with OCR and patients of control group

Rate	**Referential values**	**Patients with relapsing OC, n=41**	**Patients of control group, n=35**	**p**
Ageatthemomentofanalysis, years	Postmenopause	51.8±1.3	48.7±1.3	0.25
FSH, mIU/ml	25.2–134.8	83.0±5.3	76.3±6.1	0.15
LH, mIU/ml	7.7–58.5	40.7±2.3	36.5±2.5	0.23
E_2, pg/ml	up to 54.7	18.3±1.0	21.5±6.6	0.29
Progesterone,ng/ml	0.1–0.8	0.3±0.03	0.4±0.1	0.07
Testosterone, pg/ml	0.1–1.7	1.2±0.1	1.4±0.2	0.19

Represented results have showed that in patients with relapsing serous OC was observed high frequency of expression of SHR, HER-2/neu and VEGF in cells of primary tumor. In patients of control group, expression of SHR, HER-2/neu and VEGF was demonstrable only in cells of follicular cysts and cysts of corpus luteum of ovary, at that frequency of expression of these markers was also high. It demonstrates presence of common risk factors, hormonal pathogenesis of OC and hormone-depending benign diseases of uterus and ovary.

The highest number of estrogen-, progesterone- and testosterone-receptor-positive tumors with reliable significance was observed in patients with relapsing OC of perimenopausal period, while the lowest frequency of receptor-positive serous OC was characteristic for the patients of reproductive age. Presence of correlation between all SHR, as well as expression of ER, PR and age periods of patients with relapsing OC has been confirmed that was determined by the results of our previous studies [16]. Lack of correlation between expression of HER-2/neu, VEGF and age of patients with relapsing OC was observed that also had beenrevealed by the results of our previous studies. It points one more time at the fact that expression of HER-2/neu and VEGF in serous OC are independent factors of aggressive and unfavorable clinical course of tumor process.

The highest frequency of expression of SHR, HER-2/neu and VEGF in granulose, teca- and lutein cells of functional ovarian cysts has been determined in patients of reproductive period. Significant correlations between all SHR in cells of functional ovarian cysts; between age period of women and expression of all steroid receptors; expression of ER, PR and expression of HER-2/neu and VEGF have been established. These data confirm direct participation of expression of SHR, HER-2/neu, VEGF in granulose, teca- and lutein cells of ovary in regulation of ovarian-menstrual cycle in women [20, 21].

Significantly higher expression of SHR and HER-2/neu in cells of serous OC as compared with one in functional ovarian cysts has been identified. Our previous studies have determined that positive hormonal receptor status in patients with serous OC is a factor of unfavorable clinical course of tumor process. It allows concluding that level of expression of SHR has certain significance in clinical course of OC.

A. Kauppilaand A. Bergqvist have already demonstrated (cit. after: [17]) that samples of morphologically unchanged ovary, with benign and malignant tumor expressed all SHR. In this case, frequency of expression of ER was significantly higher in cells of OC as compared with one in benign tumor and morphologically unchanged ovaries, and for PR and TR this correlation was opposite. D. Willcocks and co-authors (cit. after: [17]) have observed low expression of ER (22%) and high expression of PR (75%) in morphologically unchanged ovaries. M. Galli and co-authors (cit. after: [17]) have determined phenotype of tumor ER+PR+TR+ in 44% of OC patients, and did not detect such phenotype in patients with benign tumor. At that positive staining of samples of morphologically unchanged ovaries for ER was observed in 46% of patients, for PR – in 54% and for TR – in 85%. L.S. Bassalyk [17] in own studies has identified similar frequency of expression of ER and PR in OC cells and cells of benign ovarian tumors, while receptors to androgens in the latter were found 15 times more often, than in cells of OC. According tothe study of V.K. Kondratyuk [34], lack of expression of ER and PR in cells of follicular and paraovarian cysts of ovary, and low receptor status in cells of endometrial cysts and cysts of corpus luteum, has been determined. Q. Mengand co-authors [35] have showed significantly high frequency and level of expression of ER-α, receptors to androgens, VEGF and COX-2 in cells of OC as compared with ones in cells of endometrial ovarian cysts.

The results of studies of V.P.Kozachenko and E.E. Makhovaya [36] have detected that levels of expression of ER, PR, receptors to androgens in tumor cells significantly are not different in menstruating women and women in postmenopause, but frequency of PR-positive OC in menstruating patients was higher and constituted 83%.S.M. Kartashov [37] have analyzed hormonal-receptor status in ovarian tumors and noted that phenotype of tumor ER+PR+ was registered significantly more often in serous OC, than in serous cystic adenomas of ovaries. In this case, mean levels of expression of ER and PR were also reliably higher in cells of serous OC. When evaluating dependence of expression of ER and PR in ovarian tumors on age of patients, author has showed that hormonal receptor status of serous OC in patients of

menopausal period was observed more often (52.2%), than in patients of reproductive period (37.5%). Such tendency was also demonstrable in patients with serous cystic adenoma of ovary.

We have determined lack of reliable differences of hormonal homeostasis in patients with relapsing OC and patients of control group, rates of which corresponded toreferential values of levels of sex hormones of postmenopausal period that allows us to assume that local concentration of gonadal hormones has certain significance in ovarian tumors.Our data contradict the results of studies of J. Wang and co-authors [38], who observed induction of expression of VEGF in serous OC, *in vitro* and *in vivo*, under the effect of increased levels of FSH and LH, having assumed that high menopausal levels of gonadotropin hormones in OC patients after surgery may accelerate progression and relapse of disease. However, results of our study adjust the data of many other studies. S. Rinaldi [39], when he analyzedthe results of large European study (192 patients with OC and 346 healthy women), and A. Lukanova [40], according to the data of their own studies, have not determined statistically significant differences in levels of testosterone, androstenedione, dehydroepiandrosteron, estron, sex-binding globulin in blood serum of OC patients and healthy women. Moreover,A. AkhmedkhanovandA.A. Arslan[28, 29] also have not detected correlation between serum levels of FSH, LH and risk of OC. S. Kramer [41] and A.Chudecka-Glaz[42],when comparing patients with benign ovarian cysts and patients with serous OC, have not determined differences in serum levels of FSH and LH, but in aspirate from serous OC they have observed reliably high levels of FSH and LH as compared with ones from benign ovarian cysts. Moreover, the results of many studies have identified that FSH- and LH-receptor complexes are able to cause hyperexpression of EGFR, in particular HER-2/neu, and via ERK½–PI3K/Akt pathway to stimulate proliferative activity of OC cells [43–45]. Activation of signal tyrosine-kinase pathway causes the stimulation of COX-1 and -2 increasing migration and invasive capability of tumor ovarian cells [44].

Estrogenization in women of postmenopausal period is known to be determined not by the level of circulating blood hormones, but by their local concentration in all tissues of organism, mostly in fat tissue. Moreover, the role of non-classical phenolsteroids and estron, which are powerful agonists of estradiol, increases in postmenopause. Activity of these metabolites increases activity of estradiol several times forming stable bounds with specific receptors of different tissues. Importance of this phenomenon consists in the fact that for occurrence and growth of tumor cells in postmenopausal women, local concentration of classical and non-classical sex steroids in tumor tissue has certain significance that is more fully studied at breast cancer [2, 33, 46, 47]. At this disease for today mechanism of interaction of steroid hormones and many other molecular markers, including HER-2/neu and VEGF, with receptor apparatus of tumor cells, and realization of receptor signals in cellular nucleus, has also been studied [48–50].

For today, mitogenetic role of estrogens in OC cells *in vivo* and *in vitro* has been proved. However, cascade signal mechanism of proliferation as the result of effect of estrogen-receptor complex in OC cells includes numerous pathways, which are not fully investigated[14].G.N. Armaiz-Pena and co-

authors [51] have demonstrated that 17-βestradiol increases expression of VEGF in OC, adhesion, migration and mitogenetic potential of ER-positive tumor and endothelial cells via MAPK. Role of progesterone and its receptors for today is not determined unambiguously [14]. Estradiol is capable to regulate level of expression of receptors of androgens, in particular, to increase it [33]. For today, it has been proved that androgen-regulating genes stimulate expression of EGFR, VEGF and cyclin-dependent kinases 2 and 4. At the same time, it has repressing effect on the expression of TGF-βand Bcl-2 [52], and dihydrotestosterone in cell lines of OC and breast cancer causes repression of p27 [53]. Moreover, M. Nourbakhshand co-authors[54] have identified activation of telomerase by testosterone and androstenedione in cell lines of OC increasing viability of tumor cells. The results of many studies have showed that pronounced expression of receptors of androgens is observed both in cell lines (OVCAR3, OSEC2) and in invasive OC, and hyperandrogenism may have risk of occurrence and progression of this disease [55, 56].

Thus, represented results are the evidence of presence of common risk factors, common pathogenetic mechanism of development of OC and hormone-dependent benign disease of uterus and ovaries that gives reason for hormone-conditionality of OC. However, complicated mechanisms of realization of hormonal-receptor signal in tumor ovarian cells, which launch processes of transformation, proliferation, invasion and metastasis, still remain not enough studied. It requires further research for substantiation of expediency of applying hormonal therapy not in cases when disease relapses, but asthe component of the primary complex treatment of patients.

CONCLUSIONS

It has been determined that in women of control group, expression of SHR, HER-2/neu and VEGF was observed only in cells of functional ovarian cysts. High frequency of expression of mentioned markers both in cells of serous OC and in cells of functional ovarian cysts was identified, but level of expression of these markers was significantly higher in serous OC.

The highest number of estrogen-, progesterone- and testosterone-receptor-positive tumors with reliable significance was demonstrable in patients with relapsing OC of perimenopause period, while the lowest frequency of receptor-positive serous OC was observed in patients or reproductive age.

Lack of correlation between expression of HER-2 /neu, VEGF and age period of patients with relapsing OC has been determined. In this case, significant correlation between expression of HER-2/neu and VEGF in cells of serous OC has been observed.

The highest frequency of expression of ER, PR, TR, HER-2/neu and VEGF in granulose, teca- and lutein cells of functional ovarian cysts has been determined in patients of reproductive period. Reliably significant correlations between all SHR in cells of functional ovarian cysts; between age period of women and expression of all steroid receptors; expression of ER, PR and expression of HER-2/neu and VEGF have been established.

Rates of hormonal homeostasis of patients with relapsing serous OC were not significantly different from ones in patients with surgical menopause, who underwent surgery on account of benign pathology of uterus, and corresponded to referential values of levels of sex hormones inpostmenopausal period.

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