NITROSATIVE STATUS IN BENIGN EPITHELIAL OVARIAN CYSTIC TUMORS OF NONENDOMETRIOID ORIGIN

INTRODUCTION
In the structure of cancer incidence special place are occupied benign tumors of the female genital organs. Ovarian tumors are in second place (6.8%) from all the female genital tumors. Benign forms are found in 75–80% of true ovarian tumors [7, 9]. Despite the fairly detailed knowledge of ovarian tumors, causes of origin and benign tumors of ovarian cysts remain open. One of the most important areas of biomedical research in this field is to elucidate the molecular mechanisms that regulate cell proliferation and apoptosis. Violation of these processes leads to increased speed and reduced cell growth differentiation characteristic of tumor cells.

ANALYSIS OF THE LITERATURE DATA AND STUDY OBJECTIVE
Recently there has been an avalanche increase in the number of scientific publications on the study of the role of nitric oxide. Three American scientists Robert F. Furchgott, Louis J. Ignarro and Ferid Murad received the 1998 Nobel Prize. The goal of researchers was to study the so-called endothelial vascular relaxation factor (endothelium-derived relaxing factor). An unexpected and important discovery was that this was a factor and nitric oxide (NO) [14].

NO is a biological messenger which is synthesized from L-arginine via nitric oxide synthase (NOS) [15]. Recent literature data show that NO, and NOS can modulate cancer-related events, including nitroative, oxidative stress, apoptosis, cell cycle, angiogenesis, invasion and metastasis [21].

It were described several forms of NOS. For the vast localization in tissues they taken to provide neuronal (nNOS), endothelial (eNOS) and macrophage (macNOS). The first two types of enzymes are preferably constitutional, and the latter operates as inducible form of NOS (iNOS) [1], and in recent times has changed somewhat classification varieties of NO-synthase including distinguished:
1) NOS type 1 (constitutional-neuronal);
2) NOS type 2 (inducible NO-synthase);
3) NOS-3 type (endothelial-constitutional).

NOS type 1 is found mainly in the structures of the central and peripheral nervous system, constantly expressed under normal and pathological, is involved in the regulation of blood pressure. NOS type 2 (iNOS) is expressed in endothelial cells and macrophages in pathological processes, particularly during inflammation, is involved in the synthesis of pro-inflammatory cytokines tumor necrosis factor α, interleukin 1β. At the same time interleukins-4, 8, 10, platelet-derived growth factor inhibiting iNOS and therefore the synthesis of NO. iNOS is expressed also in the heart at myocardial infarction, myocarditis, heart failure. iNOS was found in hepatocytes, chondrocytes. Constitutive endothelial NOS 3rd type involved in the regulation of vascular tone, expressed not only in vascular endothelium, but also in cardiomyocytes, platelets, endothelium lungs.
It were studied the stable NO metabolites in biological fluids such as blood and peripheral intracystic liquid. Aseptically peripheral blood was removed before the operation, intracystic fluid - during operation by aspiration. The collected fluid was centrifuged, stored at \( t = -70 \) °C to study. Intracystic samples of fluid from the blood impurities were excluded from the study.

To determine concentrations of stable metabolites of NO (NO\(_2\) + NO\(_3\) = NO\(_x\)) in biological fluids used the method by VA Metelskaya, NG Humanova [6]. This method is based on the recovery of NO\(_3\) to NO\(_2\) vanadium chloride (III), followed by determination of nitrite using Hrissa reagent (a solution of sulfanilamide and N-naftyletylendiamine dihydrochloride in 30% glacial acetic acid) as color image reagent (giving crimson color in the presence of NO liquids). To 0.2 ml of the sample studied fill up 0.4 ml of ethanol for deproteinized, centrifuged for 20 minutes at 3000 rotations per minute. Next to each well microplate reader was added to 80 ml of the supernatant, 80 ml of vanadium chloride and 80 ml reagent Hrissa. Measurements were made at immunosorbent analyzer Stat Fax 3200 (microplate reader) (Awareness technology Inc. Palm City, FL 34990, USA). Results are expressed in mcmol/l.

Determination of serum tumor markers CA 125, CA 19-9, HE-4 was carried out by immunochemical detection of e electrochemiluminescent method using standard reagents Roche Diagnostics (Switzerland) analyzer “Sobas®6000 analyzer series” (USA).

Operating specimens were fixed in 10% formalin before pouring in paraffin. Sections were stained with hematoxylin and eosin were reviewed and selected by a pathologist for immunohistochemical analysis. Selected deparafinized, rehydrated sections were heated in a microwave oven in 0.01 M citrate buffer (pH 6.0) for 30 minutes. The activity of endogenous peroxidase blocked with 3% hydrogen peroxide for 10 minutes, then washed with saline, phosphate buffered. Sections were incubated overnight at 4 °C with anti-iNOS rabbit polyclonal antibodies (NOS2 C-19: sc-649, Santa Cruz Biotechnology, Germany). As used conjugate avidin-biotin peroxidase solution (Dako Cytomation LSAB and system-HRP, Denmark). The signal was visualized using diaminobenzidine (Dako Cytomation Liquid DAB and substrate Chromogen System, Dako, Denmark). Sections were contrasted Harris hematoxylin, dehydrated, purified and studied morphometric. As a positive control was used a sample of skin with chronic granulomatous inflammation. The intensity of expression was assessed semiquantitative: the absence of expression – “-” with weak expression – “+”, with normal expression – “+” (corresponding to the control value), with increased expression (increased density of positive areas 30-50%) – “++”, while overexpression (increase positive half sections) – “+++” [17].

The resulting preparations were investigated using a light microscope.

Statistical analysis of the data was performed using Excel.

RESULTS AND DISCUSSION

Age of examined patients with serous cystadenomas has averaged 30.10 ± 0.51 years, with mucinous cystadenomas – 30.17 ± 0.47, with follicular cysts – 30.43 ± 0.57, with cystadenocarcinomas – 31.57 ± 31.57, in control group – 30.00 ± 0.45 and probably did not differ between groups.
The average diameter of cystic formations was the largest in the mucinous cystadenomas (11.97 ± 0.81 cm) and cystadenocarcinomas (9.90 ± 0.94 cm). In serous cystadenomas it amounted to 9.06 ± 0.60 cm, with follicular cysts 7.19 ± 0.26 cm.

Analysis of oncomarkers examined patients showed, as expected, their largest concentration in the presence cystadenocarcinomas (Table 1). In patients with follicular cysts, serous and mucinous cystadenomas the levels of oncomarkers were within reference values.

Study of NOx levels in biological fluids of examined patients revealed the likely reduction of serum neutral NO metabolites in the cystadenocarcinomas (23.1 ± 0.6 mmol/l), serous (24.2 ± 0.3 mmol/l) and mucinous (23.9 ± 0.4 mmol/l) cystadenomas in comparison with follicular cysts (25.2 ± 0.3 mmol/l) and control (26.2 ± 0.2 mmol/l) (Figure 1). Differences intracystic NOx concentrations in mucinous, serous ystadenomas and follicular cysts were statistically significant, but small. At the same time the level of NOx in intracystic contents of cystadenocarcinomas exceeded that of the follicular cysts in 1.96 times (p < 0.01), in serous cystadenomas in 1.99 times (p < 0.01) and mucinous cystadenomas in 1.79 times (p < 0.01) (Table 2, Figure 2 and 3).

**TABLE 1. LEVELS OF SERUM ONCOMARKERS IN EXAMINED PATIENTS**

<table>
<thead>
<tr>
<th>Formations’ histostructure</th>
<th>CA 125, U/ml</th>
<th>CA 19-9, U/ml</th>
<th>HE-4, pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M ± m</td>
<td>min – max</td>
<td>M ± m</td>
</tr>
<tr>
<td>Follicular cysts, n=40</td>
<td>7.32 ± 1.13k,l</td>
<td>3.20 – 29.35</td>
<td>8.77 ± 1.25k,l</td>
</tr>
<tr>
<td>Serous cystadenomas, n=60</td>
<td>24.99 ± 1.77k,l</td>
<td>3.21 – 50.50</td>
<td>22.90 ± 1.80k,l</td>
</tr>
<tr>
<td>Mucinous cystadenomas, n=60</td>
<td>17.13 ± 1.10k,l</td>
<td>7.69 – 33.21</td>
<td>28.82 ± 1.90k,l</td>
</tr>
<tr>
<td>Ovarian cystadenocarcinomas, n=30</td>
<td>137.98 ± 3.80k,l</td>
<td>32.78 – 677.52</td>
<td>51.16 ± 2.36k,l</td>
</tr>
<tr>
<td>Control group, n=30</td>
<td>6.47 ± 0.88k,l</td>
<td>0.51 – 13.56</td>
<td>11.29 ± 0.97k,l</td>
</tr>
<tr>
<td>Normative data</td>
<td>0 – 35</td>
<td>0 – 37</td>
<td>0 – 70</td>
</tr>
</tbody>
</table>

(f,l,k) significant difference with groups of women with follicular cysts, with serous cystadenomas, with mucinous cystadenomas, with cystadenocarcinomas, with control group, p < 0.05

**FIGURE 1. THE AVERAGE NOx LEVEL IN BIOLOGICAL FLUIDS OF PATIENTS (mMol/l)**

**FIGURE 2. THE INTENSITY OF iNOS IMMUNOHISTOCHEMICAL STAINING IN THE WALLS REMOVED FORMATIONS, n (%)**

<table>
<thead>
<tr>
<th>Formations’ histostructure</th>
<th>The weak intensity of iNOS expression (+/- or +)</th>
<th>Increased intensity of iNOS expression (++ or ++++)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular cysts, n = 40</td>
<td>28 (70.00)¹</td>
<td>12 (30.00)¹</td>
</tr>
<tr>
<td>Serous cystadenomas, n = 60</td>
<td>43 (71.67)¹</td>
<td>17 (28.33)¹</td>
</tr>
<tr>
<td>Mucinous cystadenomas, n = 60</td>
<td>41 (68.33)¹</td>
<td>19 (31.67)¹</td>
</tr>
<tr>
<td>Ovarian cystadenocarcinomas, n = 30</td>
<td>3 (10.00)³</td>
<td>27 (90.00)³</td>
</tr>
</tbody>
</table>

¹ (k,l) significant difference with groups of women with follicular cysts, with serous cystadenomas, with mucinous cystadenomas, with cystadenocarcinomas, with control group, p < 0.05
CONCLUSIONS

1. NO and iNOS effect of on hyperproliferation processes in the ovaries is twofold.

2. NO and iNOS reducing expression and their minor activity may impact on the cystic tumor formation and benign tumor formation in the ovaries.

REFERENCES/ЛІТЕРАТУРА


8. Raevskaya, T.A. “Donor's expression of iNOS in the high-grade cystadenocarcinoma wall. Immunohistochemistry with PAT to NOS type 2, × 300” [Image].

9. Raevskaya, T.A. “Donor's expression of iNOS in the high-grade cystadenocarcinoma wall. Immunohistochemistry with PAT to NOS type 2, × 200” [Image].


Нитрозативний статус при доброкачествених епітеліальних кістозних опухолях яєчників неендоцереміодного походження

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Серед усіх пухлин жіночих статевих органів пухлини яєчників займають друге місце (6–8%) зі співвідношенням 75–80% всіх сприйнятних пухлин яєчників. Незважача на досить радікулів діагностику, експертизи та лікування, вони надовго залишаються неясні відносно походження.

Декілька досліджень виявили важливу роль оксиду азоту (NO) у процесах малигнізації кістозних утворень. Гіперекспресія NO і підвищення активності iNOS та NOx пов’язані з процесами малігнізації епітеліальних кістозних утворень.

Ціллю проведенного дослідження стало вивчення особливостей нитрозативного статусу при доброкачествених епітеліальних кістозних пухлинах яєчників неендоцереміодного походження.

Обстежено 220 пацієнток репродуктивного віку: 40 пацієнток із фолікулярними кистами яєчників, 60 – із серозними цистаденомами, 60 – із муцинозними цистаденомами, 30 – із цистаденокарциномами неендоцереміодного походження, а також 30 пацієнток контрольної групи.

Імуногістохімічне дослідження експресії iNOS показало переважно слабкий її рівень у фолікулярних кистах і доброкачествених цистаденомах, але високий – у цистаденокарциномах.

Як показує дослідження, NO та iNOS вони та інші препарати використовують у різних стадіях фізіологічних процесів ендометрію.

Ключові слова: нитрозативний статус, кістозні утворення яєчників неендоцереміодного генезу, NOx, iNOS, імуногістохімія.