Aim of the study - to determine iron metabolism markers in with benign and malignant processes of ovaries and uterus. Study object: blood serum of 50 patients with benign and malignant processes of ovaries and uterus. Methods: clinical, morphological, immunoenzyme, immunochemiluminescent, immunoturbidimetric. Results: in the group of patients with malignant processes there were more patients with alterations of blood serum iron (46.9%), ferritin (28.2%), and transferrin (59%) concentration than in the group with benign processes, 22.3%, 11.2%, and 0%, respectively. In the blood serum of 45% of ovarian cancer (OC) patients decrease of iron concentration was determined, in 25% of patients – ferritin (FER) concentration increase was seen. It was demonstrated that number of patients with decreased transferrin (TRFER) saturation with iron is seen more often in patients with ovarian cancer of III-IV stage, than in I stage, in 95 and 75% of patients, respectively. Individual variations of all iron metabolism markers in patients with malignant and benign processes of ovaries and uterus were determined. Conclusion: alterations of iron metabolism markers indicate its more often disorders in patients with malignant processes, and they may be used in practical oncogynecology as potential markers of iron metabolism disturbances and its monitoring in the process of antitumor therapy of patients with ovarian and uterine cancer.

Key words: iron, ferritin, transferrin, transferrin saturation with iron, blood serum, benign and malignant processes of uterus and ovaries.
INTRODUCTION

Iron is considered to be one of the most important elements that are involved in metabolism of cells. Iron biological function is comprehensive: it is present in hemoglobin, myoglobin, iron-containing enzymes, and participates in oxidative and reductive reactions. Iron concentration in human blood serum depends on its resorption in gastro-intestinal tract, accumulation in intestine, spleen, bone marrow, and also on hemoglobin synthesis and disintegration and levels of its loss by organism. Iron metabolism disturbances are observed in different pathological processes, including inflammatory and necrotic processes, ulcers, infections, and tumor growth [1-4].

Ferritin (FER) is the most informative indicator of iron pool in organism. It is a main protein of deposition system for iron ions in liver, spleen, bone marrow cells and reticulocytes that changes in a number of pathological processes. Such indicator is important for diagnostics of iron deficiency or excess in human organism, especially during development of anemia, leucosis, tumor growth, acute and chronic inflammations since at mentioned pathological forms oxidation stress, cells death and iron outflow into blood serum develop. FER is also detected in extracellular fluid – blood serum, synovial fluid, and milk [5-7].

Another protein involved not only in iron metabolism but in its transport as well is transferrin (TRFER) –the protein of β1-globulin fraction, able to bind with trivalent iron ions. Iron transport into cell results from interaction of iron-TRFER complex with specific TRFER receptor of plasma membrane (TFRC - Transferrin receptor protein 1, Trfr, CD71) that is involved in iron accumulation by cell and in cell growth regulation [8-11].

To determine iron metabolism disturbances a number of iron markers in blood serum are explored –concentration of iron, FER i TRFER, and also total iron-binding serum capacity. Such complex approach is the most correct because only basing on such single marker as iron concentration in blood serum it is impossible to assess adequately the cause of disturbed iron metabolism in patient.
Raise of interest to iron metabolism disturbance in oncological patients is caused of its alterations results in tumor cells that gained importance for cancerogenesis processes comprehension. In such a way TRFER alterations were registered during mammary gland cells proliferation depending on estrogens and progesterone status [12], during cancerogenesis processes and cancer progression from Cr in situ to invasive forms of breast cancer [13, 14], and also during colorectal cancer. [15]. Iron metabolism alterations in oncogynecological patients are studied insufficiently. However knowledge about such alterations is important for doctor-gynecologist-oncologist for treatment of patients with anemias resulting from uterine bleedings at uterus body cancer or in ovarian cancer that due to its special peculiarities of clinical course is considered to be the chronic tumor process, in which pathogenesis iron metabolism disturbances also can be of importance.

**Aim** of this study was to determine peculiarities of iron metabolism in patients with cancer and benign processes of ovaries and uterus.

**OBJECTS AND METHODS OF THE STUDY**

Study object was blood serum from 50 patients with malignant and benign processes of ovaries and uterus before anticancer therapy delivery. All the patients were subject to complex examination according to standards in force, clinical diagnoses were verified on the basis of morphological examination of cervical bioptates, uterine walls scrapings, and operation material. All the patients gave informed consent about their examination results use for scientific purposes.

For iron metabolism markers investigation fasting blood from vein was collected in the morning (not less than 10 hours after last food intake). Blood serum was examined in the medical laboratory “DILA” for the number of markers, namely: iron, FER, TRFER concentrations, and TRFER saturation with iron. Iron concentration in blood serum was registered by colorimetric method with ferrosin without deproteinization (reference values with women – 6.6-26.0 mcM/l), FER – by immunochemiluminescent method (reference values – 11.0-306.8 ng/ml³), transferrin – by immunoturbidimetric method (reference values 2.0-3.6 g/l),
transferrin saturation with iron - by ratio of iron to total iron-binding capacity of transferrin (reference values - norm 22-55%). Study results were processed by statistical analysis with Student’s t-criterion determination. Markers alterations with p<0.05 were considered statistically significant.

RESULTS AND THEIR DISCUSSION
Complex clinical examination results demonstrated that of 50 patients in 32 cases malignant processes were diagnosed – in 24 ovarian cancer (OC) of I-IV stage. In 3 patients – endometrial cancer of I stage was diagnosed, in 3 – cervical cancer of I-II stage, in 2 – sarcoma of uterus, in 20 – benign processes (ovarian cysts, metrofibroma, cervical intraepithelial neoplasia). According to the results of clinical-genealogical analysis of family trees 21 patients from total number of examined women were from the families with family cancer syndrome (FCS). Patients’ distribution according to the tumor processes stage and age is presented in the Table 1. Comparative analysis of iron metabolism markers was provided in 2 groups of patients: patients with malignant and benign processes (relative control). Iron metabolism markers were compared against each other in the examined groups and with reference values for health people.

At the first phase of the study it was determined that alterations in iron metabolism markers (markers’ increase and decrease – with regard to reference values) were more pronounced among patients with malignant tumors than in patients with benign processes (Fig. 1) The following draws attention: 1) in patients both with benign and with malignant processes alterations of all examined markers were determined, with the exception of transferrin concentrations in patients with benign processes, 2) in considerable number of patients of both groups the alterations related to such a marker as TRFER saturation with iron – number of patients with alterations was 87.5% among the patients with malignant processes and 72.3% among the patients with benign processes.
Table 1.
Examined patients distribution according to the character of pathological processes and age

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Tumor process stage</th>
<th>Number of patients</th>
<th>Age variation</th>
<th>Average age/median, years</th>
<th>Number of patients with FCS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Malignant processes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>I - IV</td>
<td>24</td>
<td>33-85</td>
<td>55 / 53</td>
<td>8</td>
</tr>
<tr>
<td>Malignant tumors of uterus body and cervix (cancer, sarcoma)</td>
<td>I - III</td>
<td>8</td>
<td>45-65</td>
<td>52,5/50</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>32</td>
<td>33-85</td>
<td>51 / 53</td>
<td>11</td>
</tr>
<tr>
<td><strong>Benign processes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign ovarian cysts</td>
<td>-</td>
<td>7</td>
<td>34-70</td>
<td>52,7/ 49</td>
<td>-</td>
</tr>
<tr>
<td>Metrofibroma, endometrial hyperplasia</td>
<td>-</td>
<td>7</td>
<td>43-63</td>
<td>47 / 46</td>
<td>6</td>
</tr>
<tr>
<td>Cervical intraepithelial neoplasia (moderate and severe)</td>
<td>-</td>
<td>4</td>
<td>30-60</td>
<td>40 / 34</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>18</td>
<td>33-70</td>
<td>47,7 /48</td>
<td>10</td>
</tr>
</tbody>
</table>

Note: FCS – family cancer syndrome.
Malignant processes     Benign processes
1 – iron concentration, 2 – FER concentration, 3 – TRFER concentration, 4 – TRFER saturation with iron

Fig. 1. Distribution of patients (%) with malignant and benign processes according to alterations of iron metabolism markers comparing with norm.

In the group of patients with malignant processes there were more patients with alterations of iron (46.9%), ferritin (28.2%), and transferrin (59%) concentrations in blood serum than in the group with benign processes– 22.3%, 11.2%, and 0%, respectively, that indicates more often disturbances of iron metabolism in patients with malignant processes.

For more detailed characteristics of markers alterations individual values of iron, FER, TRFER and TRFER saturation with iron were analyzed (Table 2).
Fig. 2. Distribution of patients (%) with malignant and benign processes according to iron metabolism markers (norm, decrease or increase) in blood serum. Legends as on the Fig.1.

According to the Table 2 data at malignant and benign processes significant individual variations were registered. Characteristic feature was increase of individual values of FER concentration and decrease in TRFER saturation with iron. Average values of TRFER saturation with iron were significantly lower in patients with malignant processes comparing with patients with benign processes (12% versus 23%), i.e. almost 2 times lower (p<0.05). In general, when comparing average values of registered alterations in patients with benign and malignant processes, in later the tendency toward decrease of iron and TRFER concentrations, TRFER saturation with iron and increase of FER concentration is seen.
<table>
<thead>
<tr>
<th>Characteristics of tumor process</th>
<th>Iron concentration (4.4-27.9 mcM/l)</th>
<th>Ferritin concentration (11.0-306.8 ng/ml)</th>
<th>Transferrin concentration (2.0-3.6 g/l)</th>
<th>Transferrin saturation with iron (20-55%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant processes</td>
<td>1.9 – 13.7</td>
<td>7.0 – 1059</td>
<td>1.2 – 2.1</td>
<td>6.0 – 25.0</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>221.3</td>
<td>2.0</td>
<td>12.3</td>
</tr>
<tr>
<td>Benign processes</td>
<td>7.7 – 19</td>
<td>36.7 – 204.8</td>
<td>2.4 – 3.6</td>
<td>14.0 – 31.3</td>
</tr>
<tr>
<td></td>
<td>13.9</td>
<td>62.6</td>
<td>2.6</td>
<td>23.0</td>
</tr>
</tbody>
</table>

* Numerator – individual variations of values, denominator – average values.

To clarify the reasons of individual alterations variability the studied markers alterations were analyzed according to patients’ age. Variability in patients of different age was determined, although the age dependence was not seen, probably, on account of insufficient number of patients analyzed.

Iron metabolism markers analysis demonstrated that decrease of iron concentration was determined in 46% patients with OC. Markers alterations were analyzed depending on tumor process dissemination – in 4 patients with OC of I stage and in 20 patients with OC of III – IV stage. How it is seen from the Fig. 2, the number of patients with normal and decreased iron concentration at OC of I and III-IV stages was 50 and 45%, respectively (p>0.05).
OC I stage  
OC of III-IV stage
1 – iron concentration, 2 – FER concentration, 3 - TRFER concentration,
4 – TRFER saturation with iron.

Fig. 3. Distribution of patients (%) with OC of I and III-IV stages according
to iron metabolism markers (norm, decrease or increase).

Number of patients with increased FER concentration was the same in
patients with OC of different stages. TRFER concentration in all patients with OC
of I stage the marker was within norm limits, whereas in patients with advanced
tumor process alterations’ significant variability was seen: along with normal
values (30% of patients) marker’s increase (25% of patients) or decrease (45% of
patients) was determined. Characteristic feature was decrease in TRFER saturation
with iron for OC of III-IV stage (95% of patients), comparing with I stage (75% of
patients). This indicates more pronounced iron metabolism disturbances by such
marker as TRFER concentration in patients with advanced OC. Also the same
tendency was determined regarding TRFER saturation with iron – number of OC
patients of I and III-IV stage with decreased TRFER saturation with iron reached 75 and 95%, respectively. At all patients independently of OC stage pronounced individual variability of its markers, comparing with average data of markers examined, was determined. This indicates that studied iron metabolism markers may be applied only as individual criteria in oncological patients at their determination in dynamics or during patients’ monitoring.

Table 3.

Variations and average values of iron metabolism in blood serum of patients with OC with different tumor process dissemination*

<table>
<thead>
<tr>
<th>OC stage</th>
<th>Iron concentration ( mcM/l)</th>
<th>Ferritin concentration (ng/ml)</th>
<th>Transferrin concentration (g/l)</th>
<th>Transferrin saturation with iron (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I stage</td>
<td>3.6 – 13.7 7.0</td>
<td>7.0 – 520.0 152.5</td>
<td>1.2 – 3.1 2.2</td>
<td>6.0 – 25.0 14.2</td>
</tr>
<tr>
<td>III-IV stage</td>
<td>1.9 – 9.7 4.9</td>
<td>18.0 – 1059.0 180.6</td>
<td>1.2 – 2.6 2.0</td>
<td>3.0 – 25.0 13.8</td>
</tr>
</tbody>
</table>

*Numerator – individual variations of values, denominator – average values.

In general, the fact that in patients with malignant and benign processes of ovaries and uterus equivocal alterations in iron metabolism markers were determined represents complexity of iron homeostasis system in oncological patients that is connected not only with other metabolic systems of organism, but also with dissemination degree of tumor process and, probably, with their pathogenesis. Mechanisms of such interactions in spite of significant number of studies finally were not determined.

One of the mechanisms of iron metabolism disturbance is its redistribution in macrophage system cells that leads to decrease of iron concentration in blood serum [16]. Such condition is explained by the fact that in inflammatory processes of infectious, non-infectious or neoplastic origin macrophage system becomes more active, at that iron transfer from FER to TRFER is interrupted. FER refers to
iron-containing proteins, which major function is iron deposition and turnover. FER concentration in blood serum correlates with total FER content in human organism and serves as objective marker of iron pool in organism, since it changes depending on iron metabolism state. Low FER concentration in blood serum indicates depletion of body iron pool. FER concentration may reflect not only iron amount, but also can be manifestation of acute-phase response. However in iron deficit condition acute-phase FER response is insignificant [4, 7].

In recent years interest to FER as to oncogenic protein increased since at some malignant neoplasias (lung, stomach, breast, liver, intestinal cancer) alterations of FER were revealed in patients’ blood serum [1, 12, 13]. These data correlate with our results on more pronounced alterations in FER concentration in patients with cancer comparing with patients with benign processes. This can occur due to tumor cells necrosis and concomitant inflammation that is frequently observed at advanced cancer forms; at that iron outflow from destructing cells into extracellular space is observed. Another one more important FER characteristics is its ability to block lymphocytes, to suppress immunity in oncological patients and, in that way, to facilitate tumor growth [17]. Therefore, assumptions exist that FER concentration in oncological patients can be diagnostic marker and marker of malignancy/aggressiveness of tumor process [18, 19].

Biological significance of TRFER is iron transfer into cells. Iron amount in cells is positively related to the number of TRFER receptors CD71 on cell membrane. At substantial iron amount in cells receptors’ number decreases, whereas increased iron requirement promotes induction of TRFER receptors biosynthesis. Regulation of TRFER receptors activity occurs through increase of endo- and exocytosis rate [10]. TRFER receptors are found in lymphocytes, placenta cells, and also in tumor cells, at that when iron requirement raises the number of receptors on cell surface increases. TRFER receptors expression, i.e. iron requirement, is increased in rapidly proliferating cells, activated T- and B-lymphocytes, and macrophages [14, 18, 20]. TRFER receptors (CD71) hyperexpression was determined both in experiment (human epidermoid
carcinoma cells A431, 14 tumor cell lines of different origin), and in the cell of a number of human tumors (gliomas, cancer of pancreas, breast, and colon) [1, 12, 13, 15]. According to some data TRFER receptors expression may serve even as marker of malignant transformation of pancreatic cells – 93% of pancreatic cancer cells demonstrated positive or heterogeneous TRFER receptors expression [21]. Basing on these data it is possible to suggest that iron metabolism markers may have clinical significance and be used in practical oncology as potential diagnostic markers, as indicators of tumor process activity, and as therapeutic target.

Iron metabolism in different pathology forms draws attention of many researchers. In one of the reviews basics of iron transport conception and its reservation in cells were summarized, and also attention was drawn to the system of proteins with different function - IRE (iron-responsive element)/IRP (iron-regulatory protein), participating in regulation of iron metabolism cycle to support its homeostasis and system balance in organism [22]. Along with this, it is worth to mention that final mechanisms of such a complicated process as iron metabolism in oncological patients have not been determined yet; however, it is sound ground for further research in this direction. Until now still remains the actual determination of the role of iron metabolism disturbances not only in tumor growth pathogenesis, but also in the mechanisms of augmentation/reduction of tumor cells proliferation, oxidative stress, and also development of new approaches to oncological patients’ therapy with application of antibodies to TRFER receptors, or with iron preparation. Towards this indicate studies performed at the end of 20-th century, according to which TRFER complexes both with platinum (MPTC-63), and with other metals demonstrated cytotoxic activity and inhibition effects in cultivated tumor cells and also in animal experiments [23]. Discovery of the number of proteins (ferroprotein, hepcidin, and six transmembrane prostate antigens, proteolytic regulator of iron homeostasis matriptase-2 (TMPRSS6) [24, 25], and also family of regulator proteins of iron turnover and microRNA confirms their role in neoplastic growth and metastasing [26, 27]. Considerable significance
is devoted to further investigation of iron bioavailability, its role in cell growth and proliferation, and to genetic and molecular interaction mechanisms.

CONCLUSION

Thus, the results of conducted study show iron metabolism alterations in women with malignant and benign processes of female reproductive system organs. Irrespective of tumor growth stage in blood serum of 45% of patients with OC decrease in iron concentration, and in 25% of OC patients – increase of FER concentration were determined. In patients with OC of I stage TRFER concentration alterations were not detected, whereas in patients with IV stage OC its alterations were bidirectional: in 45% of patients – decrease, in 25% of patients – increase of markers. It was demonstrated that the number of patients with decreased TRFER saturation with iron at OC stages I and III-IV was 75 and 95%, respectively. Considerable individual variations of all iron metabolism markers were determined in patients with malignant and benign processes of ovaries and uterus that form the ground for the application of the detection of iron homeostasis disturbances in practical oncology and their monitoring in the process of antitumor therapy of OC patients.

LITERATURE


26. Greene CM, Varley RB, Lawless MW. MicroRNAs and liver cancer