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Primary cancer of the fallopian tubes: histological and immunohistochemical features

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Abstract: The rare occurrence of the fallopian tubes cancer allows to assert that the experience of even a small number of observations has a undoubted scientific and practical interest. Nowadays this type of neoplasia occurs more often in practical oncogynecology and is the primary source of serous ovarian and peritoneal tumors. The determination of tissue receptor status of primary fallopian tube cancer (ER, PR, Ki-67, HER2 / neu) will verify the degree of biological malignancy and predict the course of disease and suggest appropriate methods of treatment.

Key words: primary cancer, fallopian tubes, Ki-67, estrogen receptors, progesterone receptors.

Introduction

Despite significant progress in the study of malignant tumors, the primary cancer of the fallopian tubes (PFTC) is not fully studied yet. According to different authors its incidence accounts from 0.14% to 1.8% of all female genital malignancies [1, 2]. The causes of the fallopian tube cancer are still not clearly determined in modern gynecology. In literature there is some information that the patients with BRCA1 or BRCA 2 gene mutations are more vulnerable to this disease [3, 4]. In case of malignant tube-ovarian tumors the localization of primary neoplastic process is often difficult to

determine thus in most cases the fallopian tubes carcinoma is mistakenly diagnosed as ovarian cancer. Taking into account this fact, it can be assumed that this type of neoplasia occurs more often in clinical practice. After detailed study of infundibulum parts of the fallopian tubes R. Wilcox concluded that the ratio of PFTC of all tumors of the uterine appendages is 15% ($p < 0.001$) but not 2.3% as it was previously determined [5]. The PFTC is diagnosed if it damages the fallopian tube. This process must be dominated over the malignant process in the uterine and ovary or when its histologic structure is heterogeneous [6, 7]. Over the last decade it was proved that the fallopian tubes are the primary source of serous ovarian and peritoneal tumors [8]. Despite the ongoing discussions on the origin of ovarian, peritoneal and fallopian tubes cancer, these tumors are still classified as independent nosologic units according to international classifications [9]. Overall 5-year survival rate with PFTC is only 35% due to the rapid lymphatic spreading of the cancer [10].

The modern oncomorphology is in search of the criteria that will allow to verify the degree of biological malignancy and to predict the course of the disease with maximum objectivity. The study of molecular markers will allow to provide the adequate treatment of the patients with advanced processes and to improve the assessment of vulnerability to certain therapies. Steroid hormone receptors were among the first indicators implemented into practice and classified as cellular markers, which are determined in tumor tissue and characterize its biological properties and “behavior” [11]. Thus, the breast tumor with positive estrogen and progesterone receptor status has better survival rates than with negative receptor status and is one of the most significant independent indicators compared to other clinical characteristics of the tumor [12, 13].

Proliferative activity Ki-67 marker is successfully used in researches and diagnosing of pathologies that are accompanied by changes in cell division activity. Its expression allows to determine the “hidden” proliferative potential of the tumor, to assess its malignancy and to divide the patients into groups with relatively favorable and unfavorable course of the disease [14, 15].

Expression of oncogene Human Epidermal growth factor Receptor 2 (HER2/neu) has been also studied enough in many tumors, particularly in mammary gland. HER2/neu-positive status is often associated with a high grade of malignancy, affection of lymph nodes and recurrence tendency and indicates that the tumor is resistant to chemotherapy [16]. Assessment of the ER, PR, proliferative activity of tumor cells and expression of oncogene HER-2/neu is important not only for determination of the biological characteristics of the tumor, but also for selection the therapy.

The objective of this study is to determine the receptor status of PFTC tissue (ER, PR, Ki-67, HER2/neu).

Materials and methods

The postoperative samples from 71 women with PFTC who were treated at regional oncologic dispensaries in the period from 2005 to 2015 were used for this research. The primary nature of the fallopian tube lesions was determined according to the criteria proposed by C.Y. Hu: 1) the macroexamination showed the tumor localization in the fallopian tube; 2) microexamination revealed affection of all mucous membrane layers; 3) when the fallopian tube wall is affected during the long period, the spreading of neoplasia occurs through the tube. The stages of the disease were determined according to FIGO staging system (2009). For morphological verification of the diagnosis the paraffin blocks and histological sections, stained with hematoxylin and eosin, were used. The tumor histological type, tumor grades and degree of invasion were determined. In order to eliminate the impact of tumor histogenesis on the tumor cells immunophenotype, the criterion for inclusion in the study group of tumor was the presence of serous adenocarcinoma of the fallopian tube ($n=66$) (the cases of mucinous, clear-cell adenocarcinoma and squamous cell carcinoma were excluded).

The 3–4 μm thick sections were prepared for immunohistochemical study, which were deparaffinized and dehydrated in xylene and ethanol with increasing concentrations according to standard procedure. Antigen retrieval was conducted in water bath at 97–98°C. Antigen- antibody interaction was visualized by detection system «Ultra Vision Quanto Detection System HRP DAB Chromogen» («Thermo scientific», the USA), that included the blockage of endogenous peroxidase with hydrogen peroxide, blockage of nonspecific staining, using «Ultra V block», response intensifier «Primary Antibody Amplifier Quanto» and final visualization by diaminobenzidine with nuclei staining with Mayer's hematoxylin. To study the receptors for estrogen and progesterone the rabbit monoclonal antibodies (clone SP1 — for determination of ER, clone SP2 — for PR) were used. Assessment of ER and PR expression was carried out according to D.C. Allred recommendations (1989), taking into account the area and intensity of the nuclei staining. Presence of nuclei with positive coloring was considered as steroid-positive reaction. The reaction was considered as negative with the amount of points 0–2, weak positive — 3–4 points, medium positive — 5–6 points and strong positive — 7–8 points. To assess the proliferative activity of tumor cells the rabbit monoclonal antibodies for Ki-67 protein (Clone SP6) were used. Tumor proliferative activity was assessed by the presence of positive stained nuclei of tumor cells. Proliferation index was determined by using the microscope «MICROmed», assessing the positive reaction in 1000 cells. Ki-67 index was calculated as the ratio of specifically stained nuclei to all nuclei and were calculated in percentage: 0 points — negative reaction, 1 — weak reaction (the number of positive-stained cells (n) = 0–30%), 2 points — medium positive reaction ($30 < n > 60\%$), 3 points — strong positive reaction ($n > 60\%$). To determine the

HER-2/a neu oncoprotein the rabbit monoclonal antibodies (clone SP3) were used, taking into account the density and intensity of membrane staining. Assessment of HER2 expression was performed according to the rules DAKO Hertseptest and was classified as negative (1+) or doubtful reaction (2+).

Statistical analysis was made in Microsoft Excel 2010 with AtteStat 12.0.5. The assessment of the deflection probability of compared parameters was conducted according to Student's t-test (t). The correlation between studied parameters was assessed according to Pearson's correlation (r). The results were considered as statistically reliable if the probability level was over 95% ($r \leq 0.05$) when experimental $r \geq$ critical r.

Results

We determined that the women age affected by the fallopian tubes cancer ranges from 35 to 89 years (the mean age is 61.1 years). PFTC was most frequently observed (40.8%) in 60–69 years age group (Fig. 1).

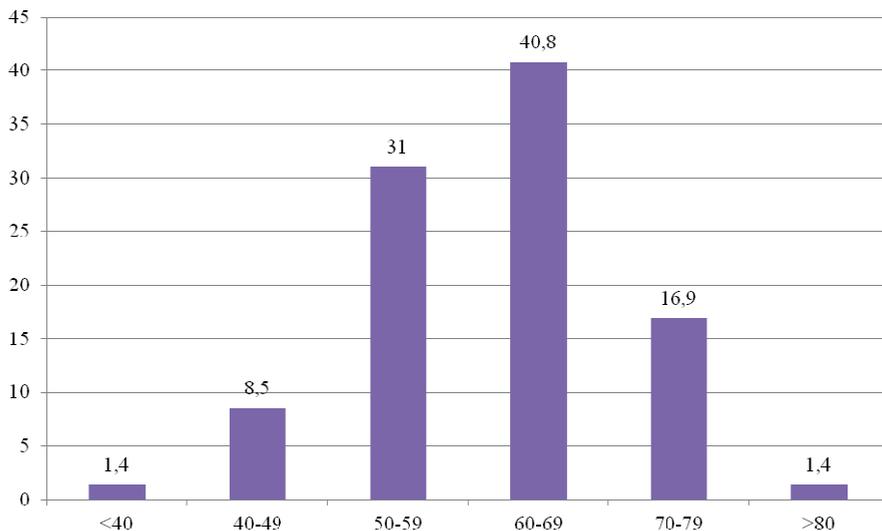


Fig. 1. Patients with primary cancer of the fallopian tubes divided into age groups.

49 (69%) of patients were postmenopausal women. As a rule the neoplastic process is the one-sided process in 83.1% ($n = 59$) and in most cases affects the ampulla of the fallopian tube. The Stage I disease was observed in 24 patients (33.8%), II — in 19 (26.8%), Stage III — in 28 (39.4%) (Fig. 2). The patients with Stage IV disease were not observed since such patients were treated only by palliative chemotherapy without surgery.

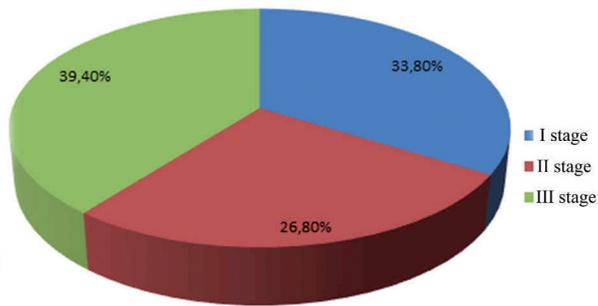


Fig. 2. Division of the patients with primary cancer of the fallopian tubes according to stages (FIGO, 2009).

All cases of serous adenocarcinoma were divided into 3 groups according to the differentiation grades: G1 — well — differentiated adenocarcinoma (low grade of malignancy) ($n = 10$), G2 — moderately — differentiated adenocarcinoma (intermediate grade of malignancy) ($n = 23$) and G3 — poorly — differentiated adenocarcinoma (high grade of malignancy) ($n = 33$).

The study of receptor status of serous cancer of the fallopian tubes showed that most of them were ER-positive (83.33%), in 41 cases (62.12%) of estrogen-positive neoplasia the expression of PR was determined. In 11 cases (16.67%) the negative profile for both receptors was observed. Cases of PR positive status of tumor cells with ER absence were not found (Table 1). However the age does not influence the phenotype of the tumor ($r = -0.09$, $p > 0.05$ for ER, $r = -0.14$, $p > 0.05$ for PR). The study of the impact of differentiation grade on the tumor receptor phenotype showed the moderate negative correlation ($r = -0.65$, $p = 1$ for ER, $r = -0.53$, $p = 1$ for PR). Table 2 shows the indicators of ER and PR expression in PFTC tissue.

Table 1. Receptor phenotype of serous adenocarcinoma of the fallopian tubes.

ER+PR+		ER+PR-		ER-PR-	
n	%	n	%	n	%
41	62.12	14	21.21	11	16.67

Table 2. ER and PR expression in PFTC tissues.

Receptor status	Total points by D.C. Allred (1989)	Number of ER cases	Number of PR cases
Negative	0–2	11	25
Weak positive	3–4	9	12
Medium positive	5–6	31	24
Strong positive	7–8	15	5

Analysis of the protein expression of Ki-67 in tumor cells of the fallopian tubes established that proliferative cells were found in all cases. The average index of proliferation was $55.02 \pm 2.73\%$ (12% to 95%). In 12 cases (18.18%) the reaction has weak positive level, in 18 cases (27.27%) has medium positive level — and in 36 cases (54.55%) has strong positive (Fig. 3).

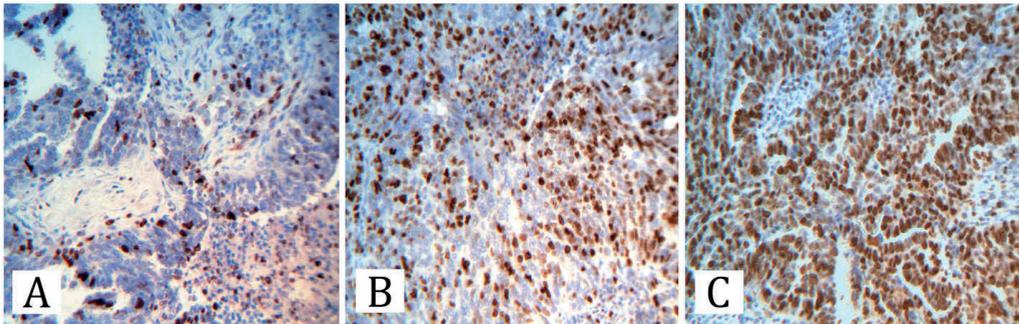


Fig. 3. Serous adenocarcinoma of the fallopian tube. A — weak positive reaction Ki-67 < 30.0%, B — medium positive reaction — Ki-67 30–60%, C — strong positive reaction — Ki-67 > 60.0%. IHC study of Ki-67 expression. Magnification $\times 160$.

The dependence of tumor proliferative activity on the patient's age was not found ($r = 0.15$, $p > 0.05$). The tumor cells proliferation didn't depend on differentiation type of serous adenocarcinomas ($r = 0.13$, $p > 0.05$).

Ki-67 expression in women with Stage I was $54.65 \pm 4.4\%$, with II — $53.94 \pm 4.76\%$, with III — $55.96 \pm 4.88\%$. No difference in Ki-67 expression of different clinical groups of tumors ($p > 0.05$) was found. Proliferative activity indicator in patients with cancer of the fallopian tube with metastases was $65.63 \pm 4.98\%$, that is significantly higher than that in patients without metastases ($50.72 \pm 3.08\%$), $p = 0.006$.

Assessment of HER-2/neu expression showed the doubtful reaction in 9.1% of cases ($n = 6$) that included moderate membrane staining — more than 10% of tumor cells and did not depend on the tumor differentiation grade ($r = 0.06$, $p > 0.05$), stage of disease ($r = 0.02$, $p > 0.05$) and presence of metastases ($r = 0.06$, $p > 0.05$).

Discussion

Since 1866, when the PFTC was firstly described (K. Orthmann), it has been considered as a rare, difficultly diagnosed disease with a bad prognosis. Stewart and others found that the incidence in women in the USA was 0.41 per 100 000 women from 1998 to 2003 [17]. Nowadays this type of neoplasia occurs more often in practical oncogynecology and is the primary source of serous ovarian and peritoneal

tumors [18, 19]. The problem of PFTC becomes more serious, although the causes and frequency of incidence, methods of diagnosis, prevention and treatment are not fully presented in modern medical literature. In patients anamnesis the infertility is rather often observed that is caused by amenorrhea or anovulatory cycles [20]. Repeated inflammations of the uterine appendages are among factors that cause the neoplasia. Some cases of the coexistence of both the PFTC and tuberculous salpingitis are described [21]. In recent years, the theory of viral etiology in the development of fallopian tube cancer is considered, particularly the role of herpes virus type II, human papillomavirus and chlamydial infection [22]. Against of the chronic inflammations in the tube, the pseudocarcinomatous hyperplasia can appear that is difficult to differentiate from PFTC and these mistakes can lead to unnecessary radical surgery [23]. Currently it is proved that carcinoma of the fallopian tubes should be considered as part of the hereditary breast and ovarian cancer syndrome (HBOC) and may be connected with BRCA1 and BRCA2 gene mutations [3, 4, 24]. There are some facts about women with BRCA1/2 mutations that prove that infundibulum parts of the fallopian tubes is an important place of cancer occurrence [25–27].

Preoperative diagnosis of PFTC is usually difficult. Such symptoms as watery vaginal discharge, pain in the lower part of abdomen and extended mass in the pelvic on the side of the uterine are observed only in 15% of cases [28]. The most of patients with PFTC undergo the laparotomy with the presumed diagnosis of the ovarian cancer due to the presence of adnexal mass in pelvic. Thus, the PFTC should always be included in the differential diagnosis. High disposition for lymphatic metastasis is explained by abundant lymphatic network in the fallopian tubes. Considering this fact, there is an offer to remove the pelvic and paraaortic lymph nodes [29–31]. The results of this study demonstrate that mean age of patients is 61.1 years (from 35 to 89 years), that corresponds to the previous studies [32], although sometimes PFTC is observed in young women (17–19 years) [28]. In 92.96% of cases the serous adenocarcinoma with various grades of differentiation was observed. The study of receptor status of serous cancer of the fallopian tubes showed that the majority of them are receptor — positive to both receptors or to ER-receptor (83.33%). Only in 62.12% of cases the PR positive reaction was observed, that determine the tumor vulnerability to hormonal therapy. The cases of PR positive tumor cells with the absence of ER were not found, that prove the dependence of PR on the sensitivity of tumor cells to estradiol. Thus, the receptor profile of fallopian tubes tumor did not depend on the age. When the malignancy increases the number of receptors for steroid hormones is reduced. The analysis of Ki-67 expression showed any correlation between indicators of proliferative activity, grades of differentiation and disease stages that indicates the independence of the speed of cell division in neoplasia, although in the majority of malignant tumors this indicator had direct link [33]. Proliferative activity in the tumors of the fallopian tubes with metastases was significantly higher

than in tumors without metastases. It means that the study of Ki-67 expression is independent prognostic marker that should be considered in diagnosing the PFTC. This indicator has more significant impact on the spreading of neoplasia through the body than its histological characteristics.

The analysis of oncoprotein HER-2/neu expression in tissue of PFTC showed the doubtful reaction in 9.1% of cases ($n = 6$) that significantly differs from the studies of other authors who describe its high expression in more than 30% [34–36] but didn't connect it with its presence and the survival rate of women. This may be caused by both the ethnic peculiarities of the patients and the tumor sensitivity to different antibodies (depends on the producer). This research demonstrates that fallopian tubes cancer is mainly HER-2/neu negative and we cannot recommend this oncoprotein in routine examinations of such patients.

Conclusions

Fallopian tubes pathologies require proper attention of both pathologists and gynecologists. The primary cancer of the fallopian tubes more commonly affect the postmenopausal women, aged 60–69 years, mainly in early stages of the disease (I–II) (60.8%) and in most cases was represented as serous adenocarcinoma (92.96%). It was determined that most of them are receptor-positive for both steroid receptors (ER — 83.33%, PR — 62.12%). But the receptor profile of the tumor of the fallopian tubes did not depend on the age. When the tumor differentiation grade becomes lower the number of receptors for steroid hormones also reduces. High level of proliferative activity is typical for this type of neoplasia. This activity is not depend on the age, stage of the disease and tumor differentiation grade. But it indicates the severity course of the disease that influences the spreading of neoplasia through the body. Considering this fact the Ki-67 expression is independent marker for N-status and helps to determine the patients who are in the “risk” group. HER-2/neu expression is not typical for primary cancer of the fallopian tubes, taking into account almost complete lack of it (only 9% — doubtful reaction) in tumor tissue.

Conflict of interest

None declare.

Authors' contributions

All authors agreed to be accountable for all aspects of the work and ensuring accuracy and integrity and approved the final version of this manuscript.

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