Original article

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CLINICAL-PATHOLOGICAL CHARACTERISTICS OF HORMONE INDEPENDENT LOBULAR BREAST CARCINOMA

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Estrogen has a role in the proliferation of luminal layer of epithelial breast cells and approximately 70% of human breast cancers have estrogen receptor expression. Based on the hormone receptor expression, we can classify these carcinomas as hormone-dependent and hormone-independent. Considering that the data in the world literature are incomplete, the aim of this research was a comparative analysis of these characteristics of hormone-dependent and hormone-independent lobular breast carcinomas. One hundred thirty-eight cases of lobular breast carcinomas were analyzed in relation to their hormonal status. Obtained morphometric values were subjected to statistical analysis using Student's t-test and Fisher's test. Statistically significant difference between groups of patients with hormone-dependent and hormone-independent lobular breast carcinomas was found for the age of patients ($p = 0.036$) and nuclear gradus ($p = 0.006$). On the other hand there was no statistically significant difference between two groups of patients considering the presence of metastasis in the axillary lymph nodes ($p > 0.05$). It was found that the patients with hormone-independent lobular breast carcinoma were significantly older then the patients with hormone-independent lobular breast carcinoma, and that expression of hormone receptors did not play a key role in metastasis of this carcinoma to the axillary lymph nodes.

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Key words: *hormone dependence, lobular breast carcinoma, lymph node status*

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Introduction

Every year, about a million women in the world are diagnosed with breast cancer. Estrogen plays a role in the proliferation of the luminal layer of breast epithelial cells and the development of breast cancer. About 70% of human breast cancers show estrogen receptor expression (1, 2). Breast cancers have long been classified on the basis of numerous requirements as estrogen-dependent or estrogen-independent tumors (3). Estrogen is a transcription factor that regulates the genetic program of cell cycle progression and growth in the breast glands (2).

For more than three decades, ER has been the most important biomarker for breast cancer treatment, primarily because of the significant benefits of endocrine therapy for ER-positive but not for ER-negative tumors in women of all ages (4, 5).

The development of immunohistochemical methods using monoclonal antibodies has enabled the determination of hormone receptors, which is of particular importance for determining the biological potential of breast cancer. Assessment of hormone receptor expression in the nuclei of breast cancer tumor cells is a part of routine diagnosis and provides important information on prognosis and relevant therapeutic approach (6).

Hormone therapy reduces the relative risk of disease recurrence in more than 50% of patients with hormone-dependent breast cancer (7-9).

The aim

Since the data in the world literature are partial, a comparative analysis of the clinical and pathological characteristics of hormone-dependent and independent lobular breast cancers has been set as the goal of this research.

Materials and methods

Twenty-eight cases of lobular breast cancers diagnosed in the period from 2007-2009 were analyzed. The other 110 selected cases of lobular carcinomas that occurred in the same period were taken for the purpose of mutual comparison. Tissue samples of lobular breast cancers, obtained by excisional biopsies or mastectomy with axillary dissection, were used for this study. It is important to mention that all these patients were treated at the University Clinical Centre Niš. Samples were routinely processed in paraffin molds and archived together with pathohistological and clinical documentation at the Institute of Pathology of the University Clinical Center in Niš.

Paraffin molds were microtomically cut into sections of about 4 μm in thickness, adhered to "super frost" plates, and stained immunohistochemically for estrogen receptors (ER) and progesterone receptors (PR). After dewaxing and hydration of the samples through xylene and a series of decreasing concentrations of alcohol, the antigen was unmasked in a microwave oven, 20 min in citrate buffer, followed by cooling to room temperature, washing and blocking endogenous peroxidase with 3% hydrogen.

The samples were washed in PBS buffer (Phosphate Buffered Saline), pH = 7.4, and then the primary antibody was applied with an incubation of 40 min at room temperature. PBS lavage was followed by Labelled Streptavidin-Biotin 2 System, Horseradish Peroxidase (LSAB2 System-HRP, 15 ml, Code K0673), containing yellow and red LINK and incubated for 20 min, and PBS-lavage was performed between each step. Visualization was performed with DAB (Diaminobenzidine), followed by good rinsing with running water for 2 min, hematoxylin contrast, dehydration, and tissue incorporation with DPX. DAKO (Glostrup, Denmark) reagents were used.

The results of immunohistochemical analyzes of ER and PR expression were evaluated semi-quantitatively based on the Allred "scoring" system, where the total score was obtained by: sum of percentage involvement of tumor cell nuclei (score 1 - less than 1% of tumor cell nuclei; score 2 - of 1 up to 10% of tumor cell nuclei, score 3 - from 11 to 33% of tumor cell nuclei, score 4 - from 34 to 66% of tumor cell nuclei, score 5 - more than 67% of tumor cell nuclei) and score of nuclear staining intensity - weak, score 2 - medium, score 3 - strong). Tumors with a score of 2 or more were considered positive.

Patients with metastatic disease of the visceral organs, as well as patients with incomplete hormone-dependent lobular carcinomas (ER +/PR - and ER -/PR +) were not analyzed.

The obtained values for the examined parameters, such as age of patients, pT stage, histological grade, nuclear grade, axillary lymph node involvement in hormone-independent and hormone-dependent lobular breast cancers were subjected to the following methods: descriptive statistics (average value and standard deviation) and comparative tests (parametric (t-test) and non-parametric (Fisher's test)) type using GraphPad Prism version 5.03 (San Diego, CA, USA).

Results

Out of 138 examined patients with lobular breast cancer, 28 were not hormone dependent and 110 were hormone dependent. The average age of patients, as well as the average age of patients with ER and PR positive and negative scores are shown in Table 1. Based on the student's t-test, a statistically significant difference was found in the average age of patients with hormone-independent or hormone-dependent lobular cancer. The absence of immunohistochemical staining of tumor cell nuclei in hormone-independent lobular breast cancers is given in Figure 1. No statistically significant relation was observed for pT stage and histological grade of hormone-dependent and hormone-independent lobular breast cancers. Patients with hormone-independent lobular carcinoma of the breast (Figure 2) were statistically significantly more likely to have a high nuclear grade (NG III) compared to patients with hormone-dependent lobular carcinoma of the breast (60% vs. 28.18%), and significantly lower incidence of grade I and grade II (7.14% vs. 13.63% and 32.14% vs. 58.18%) (Fischer test, $p = 0.006$) (Table 1).

Statistical analysis of the relationship between the presence of metastases in axillary lymph nodes and hormonal status of lobular breast cancer showed no statistically significant difference (Fisher's test, $p = 0.187$), where patients with hormone-independent LCD were more likely to have metastases in axillary lymph nodes compared to patients with hormone-dependent LCD (25% vs. 21.8%) ($p > 0.05$) (Table 2).

Table 1. Average age of patients, pT stage, histological grade, nuclear grade of patients with hormone-independent and hormone-dependent lobular breast cancers

Parameter/Hormonal type LCD	**Hormone-independent**	**Hormone-dependent**	***p* value**
Number of cases	28	110	/
Average age	53.3 ± 9.9	51.1 ± 10.3	0.036
pT stage			
pT1	13	60	0.4561
pT2	10	35	
pT3	4	7	
pT4	1	8	
Histological grade			
I	2	9	0.1120
II	9	58	
III	17	43	
Nuclear grade			
I	2	15	0.006
II	9	64	
III	17	31	

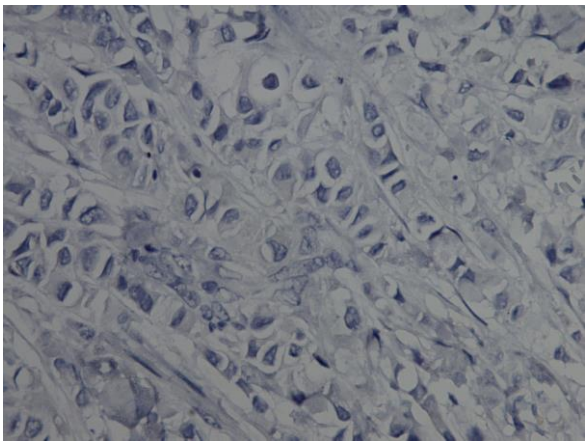

Figure 1. Negative estrogen receptor expression in the nuclei of hormone-independent lobular breast carcinoma (LSAB x 400)

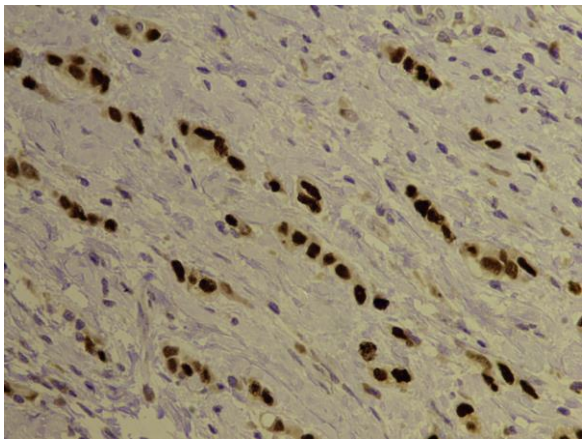

Figure 2. Positive progesterone receptor expression in hormone-independent lobular breast carcinoma (LSAB x 400)

Table 2. Relationship between hormone-dependent and hormone-independent lobular carcinomas and the presence of metastases in axillary lymph nodes.

Lymph node status	**Hormone-independent**	**Hormone-dependent**	Total	*p* value
Positive	7	24	31	0.187
Negative	21	86	107	
Total	28	110	138	

Discussion

Assessment of hormone receptor expression in the nuclei of breast cancer tumor cells is a part of routine diagnosis and provides important information on prognosis and relevant therapeutic approach (5, 6).

It is recommended that ER and PR receptor analyses should be considered positive if there is at least 1% of positive tumor cells on the tested samples in the presence of expected reactivity of internal (normal epithelial elements) and external control. The absence of benefits from endocrine therapy in women with ER-negative invasive breast cancer has been confirmed by detailed reviews of randomized clinical trials. Tumors showing less than 1% of positively stained tumor cell nuclei for ER or PR of any intensity should be considered negative, based on data that such patients do not benefit significantly from endocrine therapy (10).

Estrogen stimulates the growth and differentiation of ductal epithelium and the growth of intralobular connective tissue (11). Terminal ductulo/lobular breast units in young women are much more sensitive to estrogen, which has histamine-like effects on breast microcirculation, and the statistically significantly lower average age was in this study in patients with hormone-dependent lobular breast cancer ($p = 0.036$).

Although hormone receptor expression is associated with better ILC differentiation and lower nuclear grade, it can also be considered as an independent prognostic factor (12), which is consistent with our results that patients with hormone-independent lobular breast cancer statistically significantly more often have a high nuclear grade ($p = 0.006$). More importantly, however, ER and PR receptor expression is the most reliable predictive factor in response to endocrine therapy in breast cancer.

Some studies of patients with different subtypes of ILC do not show a significant difference in age, tumor size, growth pattern, lymph node status, and immunohistochemical expression of hormone receptors (13).

It is also considered that the prognostic value of standard pathological variables, such as tumor size, tumor grade and lymph node status, is significantly reduced in these patients with negative hormone receptors (14).

On the other hand, the importance of lymph node status in these patients cannot be determined with certainty, given that there are results that indicate the (non) existence of positive or negative correlation between negative expression of hormone receptors and lymph node status, Brouckaert et al., 2012). In this study, no statistically significant difference was found between the status of lymph nodes in patients with positive or negative expression of hormone receptors in lobular breast cancer ($p > 0.05$). Such results are possibly a consequence of the omission of patients with visceral metastases, as well as patients with incomplete hormone-dependent lobular carcinomas (ER +/PR - and ER -/PR +), which according to some authors may benefit from antihormone therapy (15).

Conclusion

By analyzing the clinical and pathological characteristics of hormone-dependent and hormone-independent lobular breast cancers in 138 patients, the following conclusions were drawn:

1. Patients with hormone-independent lobular breast carcinomas are significantly older than patients with hormone-dependent lobular breast carcinomas.
2. Patients with hormone-independent lobular breast carcinomas are statistically significantly more likely to have a high nuclear grade.
3. There is no significant difference between hormone-dependent and hormone-independent lobular breast carcinomas of patients in relation to the presence of metastases in axillary lymph nodes.
4. Understanding the relationship between hormone-dependent and hormone-independent lobular breast cancers in future studies could help find new targets in clinical "image" and pathohistological diagnosis, as well as drug development.

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KLINIČKO-PATOLOŠKE KARAKTERISTIKE HORMONSKI NEZAVISNIH LOBULARNIH KARCINOMA DOJKE

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Estrogen igra ulogu u proliferaciji luminalnog sloja epitelnih ćelija dojke i u razvoju karcinoma dojke. Oko 70% humanih karcinoma dojke pokazuje ekspresiju estrogenskih receptora. Na osnovu ekspresije hormonskih receptora, ovi karcinomi mogu se podeliti na hormonski zavisne i hormonski nezavisne. S obzirom na to da su podaci u svetskoj literaturi parcijalni, kao cilj ovog istraživanja postavljena je uporedna analiza ovih karakteristika hormonski zavisni i hormonski nezavisnih lobularnih karcinoma dojke. Analizirano je 138 slučajeva lobularnih karcinoma dojke u odnosu na njihov hormonski status. Dobijeni patohistološki, imunohistohemijski i klinički podaci upoređeni su studentovim t-testom i Fisherovim testom. Statistički značajne razlike između grupa bolesnica sa hormonski zavisnim i hormonski nezavisnim lobularnim karcinomom dojke pronađena je za starost bolesnica ($p = 0,036$) i nuklearni gradus ($p = 0,006$). Dok, sa druge strane, ne postoji statistička razlika između dve ispitivane grupe bolesnica u pogledu prisustva metastaza u aksilarnim limfnim čvorovima ($p > 0,05$). Nađeno je to da su bolesnice sa hormonski nezavisnim lobularnim karcinomima značajno starije od bolesnica sa hormonski zavisnim lobularnim karcinomom dojke, kao i da ekspresija hormonskih receptorane igra ključnu ulogu u metastaziranju ovog karcinoma u aksilarne limfne čvorove.

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Ključne reči: *hormonska zavisnost, lobularni karcinom dojke, status limfnih čvorova*