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The concentration of matrix metalloproteinase 9 in the tumor and peritumoral tissue as a prognostic marker in the breast cancer patients

Koncentracija matriks metaloproteinaze 9 u tumoru i peritumorskom tkivu kao prognostički marker kod bolesnica sa karcinomom dojke

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Abstract

Background/Aim. Breast cancer is one of the most common malignancies among women all over the world. Tumor microenvironment represents one of the main regulators of tumorigenesis. We investigated the role of matrix metalloproteinases 9 (MMP-9) concentration in peritumoral tissue as a prognostic marker in the breast cancer patients. **Methods.** The ELISA test was used to determine a total MMP-9 concentration in carcinoma and peritumoral tissue sample in the patients with breast cancer. Comparison of MMP-9 protein expression with the clinicopathological parameters was evaluated. **Results.** Peritumoral tissue at 3 cm distance from the tumor produces more MMP-9 than the tumor itself. The ratio of concentrations of MMP-9 in the tumor and peritumoral tissue considerably changes in favor of peritumoral tissue with the increase of tumor size and the involvement of axillary lymph nodes. In N0 stage, the concentration ratio of MMP-9 in the tumor and peritumoral tissues was 1 : 1.44, but in the N2 stage, the ratio was 1 : 26.5. **Conclusion.** In patients with breast cancer even in an early stadium there is a change in MMP-9 concentration in peritumoral tissue. We can extract the group of patients at increased risk for the development of lymph node metastasis. A statistically significant difference between the concentrations of MMP-9 in the peritumoral tissue and cancer tissue exists only in case of metastatic disease not in MO stadium implying need for early detection of still unknown metastases in such patients.

Key words:
breast neoplasms; disease progression; matrix metalloproteinase 9; risk assessment; tissues.

Apstrakt

Uvod/Cilj. Karcinom dojke je jedna od najčešćih malignih bolesti žena širom sveta. Tumorsko mikrookruženje predstavlja jedan od glavnih regulatora tumorogeneze. Istraživali smo ulogu koncentracije matriks metaloproteinaze 9 (MMP-9) u peritumorskom tkivu kao prognostičkog markera kod bolesnica sa karcinomom dojke. **Metode.** ELISA test je korišćen za određivanje koncentracije ukupne MMP-9 u uzorcima karcinomskog i peritumorskog tkiva kod bolesnica sa karcinomom dojke. Vršeno je poređenje proteinske ekspresije MMP-9 sa kliničko-patološkim parametrima. **Rezultati.** Peritumorsko tkivo na distanci od 3 cm od tumora produkovalo je više MMP-9 nego sam tumor. Odnos koncentracija MMP-9 u tumorskom i peritumorskom tkivu se znatno menjalo u korist peritumorskog tkiva sa porastom veličine tumora i obimom zahvaćenosti aksilarnih limfnih nodusa. U N0 stadijumu odnos koncentracija MMP-9 u tumorskom i peritumorskom tkivu bio je 1 : 1,44, dok je u N2 stadijumu bolesti taj odnos bio čak 1 : 26,5. **Zaključak.** Kod bolesnica sa karcinomom dojke čak i u ranom stadijumu postoje promene u koncentraciji MMP-9 u peritumorskom tkivu koje mogu poslužiti kao prognostički parametar u proceni agresivnosti bolesti. Analizom odnosa MMP-9 u

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tumorskom i peritumorskom tkivu možemo izdvojiti grupu bolesnica koja je pod povećanim rizikom od razvoja limfonodalnih metastaza. Statistički značajna razlika između koncentracija MMP-9 u peritumorskom tkivu i tkivu karcinoma postoji samo u slučaju metastatske bolesti, ali ne u M0 stadijumu, ukazujući na potrebu za ranom detekcijom još uvek nedijagnostikovanih metastaza kod takvih bolesnica.

Ključne reči:
dojka, neoplazme; bolest, progresija; matriks, metaloproteinaze 9; rizik, procena; tkiva.

Introduction

Breast cancer is one of the most common malignancies among women all over the world. Breast cancer mortality in the European Union is 58,000 women a year with an estimation of 135,000 new patients diagnosed every year [1]. According to the data from 2012, breast cancer morbidity in Central Serbia was 26% of all malignant diseases [2]. Although breast tumors can appear at early age, the incidence is much higher after the 5th decade and is connected with hormonal changes [3].

Tumor environment (microenvironment, or peritumoral tissue) represents one of the main regulators of tumorigenesis containing cancer-associated fibroblasts having the role in the synthesis of proteins that can remodel extracellular matrix [4, 5]. It is well-known that matrix metalloproteinase-9 (MMP-9) can be secreted from the cancer stromal fibroblasts and endothelial cells [6, 7]. The metastasis of primary tumors depends not only on cancer cells themselves, but the microenvironment as well [8]. Peritumoral tissue must not be considered as a static organ for energy storage, but as an active factor in the communication between the tumor itself and its microenvironment, producing many cytokines, growth factors and hormones that can affect the tumor growth and development [9]. Matrix metalloproteinases (MMPs) is also known as a gelatinase B and is involved in the degradation of extracellular matrix and type IV collagen, the main component of basement membrane [10–13]. It promotes cancer progression by increasing the cancer cell proliferation, migration, invasion, metastasis and angiogenesis [14]. Numerous papers suggest that MMP-9 could be used as a good prognostic marker [15]. MMP-9 protein is located in the tumor cell cytoplasm, but in the stromal fibroblast cells as well, representing one of the tumor microenvironment components [16]. Although the tumor cells are the ones breaking several tissue barriers with the aim of proliferation and spreading, there is some evidence suggesting that the tumor microenvironment cells, such as stromal cells, support the processes of tumor progression [17–19]. The findings suggested that MMPs overexpression might be associated with poor prognosis in breast cancer and its expression is increased with the increase of tumor stage [20–23]. Appropriate resection of cancer considers macroscopically and microscopically clean margins of specimen. We hypothesized that despite the absence of malignant cells, there are some parameters in peritumoral tissue, which can, on a molecular level, indicate a biologically aggressive tumor with poor outcome. Therefore, we performed the study to explore the concentration of MMP-9 in the invasive breast cancer and peritumoral tissue and its value as a prognostic marker. The novelty in our work was the analysis of ratio of MMP-9 concentration between the tumor and peritumoral tissues as a prognostic parameter in the patients with the breast cancer.

Methods

Tissue collection and processing

This work was performed in compliance with the Helsinki Declaration. Both carcinoma and surrounding peritumoral tissue were analyzed. After the decision made by the medical specialists on the combined meeting of surgeons, oncologists and radiologists, an indication for surgery was achieved. All patients who were eligible for surgery were included in the study. After approval of the Ethics Committee of Clinical Centre in Kragujevac (CC Kragujevac No. 01-4990) and written patients' informed consent, the samples were collected from 50 patients. During the surgery regularly performed at the General and Thoracic Surgery Department in Kragujevac, the samples of breast cancer tissue and peritumoral tissue were collected from each patient. The samples of carcinoma tissue were different in size, depending on the size of the tumor itself, whereas the samples of peritumoral, macroscopic unchanged tissue were taken at 3 cm distance from the macroscopic tumor margin. All the samples were histopathological verified by the Department for Pathological and Anatomical Diagnostics in Kragujevac. Weight of samples was measured and they were stored at the temperature of -196ºC until using. All samples were frozen after the initial histopathologic and immunohistochemical analysis after the surgery and all at once thawed and analyzed. The cases were evaluated for the histological type, tumor grade, histological grade (according to the Nottingham histological scores), patient age, lymph node metastasis and estrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER2/neu) status, according to the American Joint Committee on Cancer (AJCC, 7th ed., 2010) [24, 25].

The study excluded the patients with preoperatively conducted neoadjuvant therapy. The study excluded the patients with previous breast cancer history. Metastatic tumors from other tissue origins were excluded.

Tissue sample preparation

The sample preparation was performed on ice. Homogenization was conducted by adding 500 µl lysis buffer for 0.01 g sample. The IKA Homogenizers IKA®-Werke GmbH & Co. KG, Germany and Ultrasonic homogenizers Sonopuls, BANDELIN electronic GmbH & Co, Germany were used for the automatic sample homogenization. Lysis buffer contained the following components: 31.25 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol and filled with up to 100 mL dH2O. After 10-min centrifugation at 10,000 rpm at 4°C, supernatant, representing the total cell lysis, was isolated. In this manner, total proteins

from the carcinoma and peritumoral tissue were isolated and the total protein concentration in supernatant was determined. Supernatants were aliquoted and preserved at -80°C until used.

Protein concentration determination

Concentrations of protein in supernatant (samples of carcinoma and peritumoral tissue) were determined using the Lowry method [26].

Determination of metalloproteinases 9 concentration (MMP-9)

Concentration of total MMP-9 (human MMP-9 assay measures the 92 kDa Pro-MMP-9 and the 82 kDa active MMP-9) was detected by the immune/sandwich ELISA method according to the kit procedure (Human MMP-9, R&D Systems). The method is based on the measurement of total MMP-9 amount, the enzyme included in degradation of extracellular matrix which could be in active form or preform [27]. After determination, the total protein concentration, supernatants of peritumoral and carcinoma tissue were used as the samples for the total MMP-9 concentration. The MMP-9 concentration in ng/mL (*per* mg proteins) was calculated according to the known concentrations of MMP-9 from a standard curve.

Statistics

The data were analyzed by using the SPSS (ver. 13.0, Chicago, IL, USA). The data were expressed as mean (four biological replicates) and the standard error of mean (SEM). The unpaired Student's t-test, ANOVA, abnormal χ^2 test, Mann–Whitney *U* tests, Kruskal-Wallis H tests were used. The correlations between two variables were assessed using the Spearman's rho test. All statistical tests were two-sided and the *p* values of 0.05 were considered as statistically significant.

Results

The clinical and pathological parameters of patients with the breast cancer such as margin purity, histological type, histological grade, tumor size, lymph node infiltration, presence of distant metastases (M), receptor status for ER, PR and HER, patient age are given in Table 1.

Figure 1 represents the values of the total MMP-9 concentrations in peritumoral and carcinoma tissue individually for each patient. After comparing the total MMP-9 concentrations, it was indicated that the microenvironment of carcinoma tissue in most samples (72%) produced higher concentrations of MMP-9 than carcinoma tissue (28%).

The mean concentration value of total MMP-9 in peritumoral tissue was significantly higher compared to the concentration in carcinoma breast tissue (Table 2). The correlation between production of total MMP-9 in the carcinoma and peritumoral tissue in the breast cancer patients was statistically significant indicating that the increase of MMP-9 production in carcinoma tissue was associated with the increase of MMP-9 production in peritumoral tissue (Spearman's correlation coefficient, $r = 0.612$, $^{**}p < 0.01$).

The concentration of total MMP-9 in peritumoral and carcinoma tissue of breast cancer was analyzed and compared to margin "cleanness" (Figure 2). The study showed that the highest percentage of samples had clean margins on macroscopic and microscopic examination or R0 resection without the residual carcinoma cells (96% of the total number of samples).

Table 1

Clinical and pathological characteristics of breast cancer patients

Characteristics	Patients, number (%)
Samples	
peritumoral tissue	50 (50)
cancerous tissue	50 (50)
Resection (R) margin	
R0 – no residual tumor	48 (96)
R1 – microscopic residual tumor	2 (4)
R2 – macroscopic residual tumor	0
Histological type of tumor	
invasive ductal carcinoma	43 (86)
invasive lobular carcinoma	7 (14)
Histological grade (G)	
G1 – well differentiated (low grade)	6 (12)
G2 – moderately differentiated (intermediate grade)	28 (56)
G3 – poorly differentiated (high grade)	16 (32)
G4 – undifferentiated (high grade)	0
Tumor (T) size	
T1 (≤ 2)	21 (42)
T2 (2–5)	27 (54)
T3 (> 5)	2 (4)
T4 (tumor extends to skin or chest wall)	0
Age (years)	
< 40	2 (4)
> 40	48 (96)

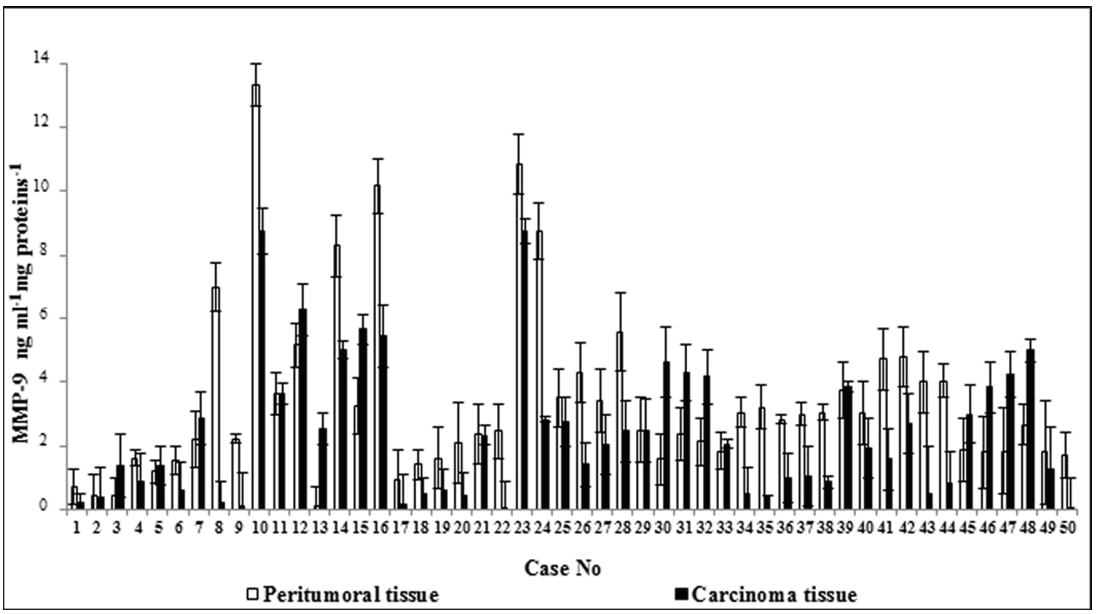

Fig. 1 – Concentrations of total matrix metalloproteinase 9 in carcinoma and peritumoral tissue in the breast cancer patients.
The data were expressed as mean ± standard error (SE) for four independent measurements of each sample.

Table 2

Concentrations of total matrix metalloproteinase 9 (MMP-9) *vs* clinical and pathological characteristics of breast cancer patients

Concentrations of total MMP-9	Peritumoral tissue (mean ± SD)	Carcinoma tissue (mean ± SD)
Mean	3.41 ± 0.27	2.40 ± 0.42*
Invasive ductal carcinoma	3.39 ± 0.31	2.40 ± 0.24*
Invasive lobular carcinoma	3.42 ± 0.27	2.45 ± 0.21*
Age (years)		
< 40	4.61 ± 0.33	3.95 ± 0.58
> 40	3.37 ± 0.28	2.95 ± 0.21

***p < 0.05 statistically significant difference carcinoma *vs* peritumoral tissue.**

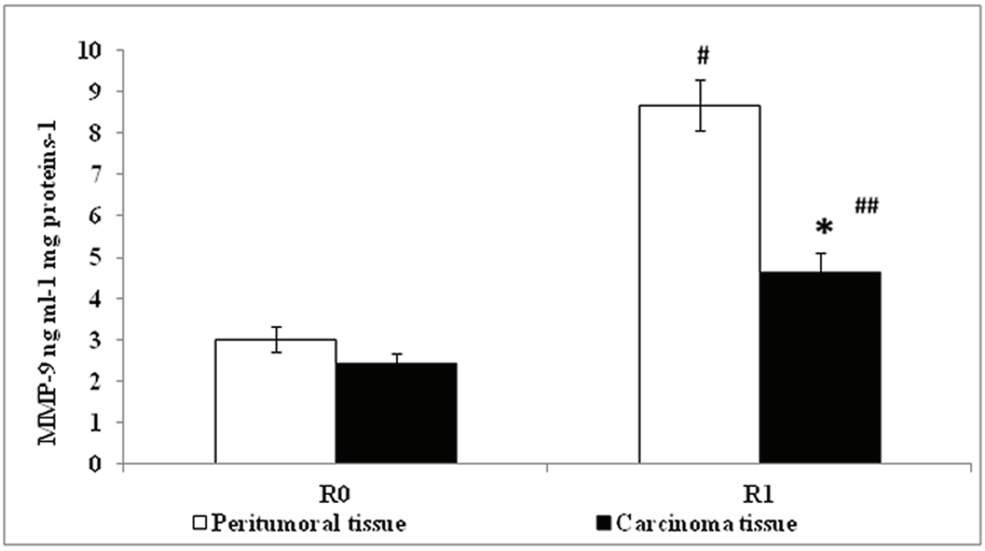

Fig. 2 – Concentration of total matrix metalloproteinase 9 (MMP-9) in peritumoral and carcinoma tissue in the breast cancer in relation to clean margins (R0) and tumor cell affected margins (R1).
The data were expressed as mean ± standard error (SE); n = the number of samples with R0 and R1; *p < 0.05 statistically significant difference carcinoma *vs* peritumoral tissue; #p < 0.05 statistically significant difference R1 *vs* R0 in peritumoral tissue; ##p < 0.05 statistically significant difference R1 *vs* R0 in carcinoma tissue.

Peritumoral tissue around ductal and lobular carcinoma type produced statistically significant higher concentrations of MMP-9 compared to carcinoma tissue (Table 2). The difference in production of MMP-9 in the carcinoma and peritumoral tissue was statistically significant in the ductal and lobular carcinoma type.

It was found that peritumoral tissue around the tumor with the histological grade G3 produced statistically higher concentration of total MMP-9 compared to carcinoma tissue (Figure 3). Amount of MMP-9 was proportionally growing in peritumoral tissue with the increase of histological grade. Peritumoral tissue around the cancer with the high grade (G3) produced statistically higher MMP-9 concentrations compared to other groups.

Figure 4 represents the concentration and distribution of MMP-9 in relation to the TNM classification (Figure 4). The results showed that the MMP-9 concentration was statistically higher in peritumoral tissue around the tumor of the largest dimensions (T3). At the same time, the tumor T3 produced statistically considerably smaller amounts of MMP-9 compared to the small dimension tumors.

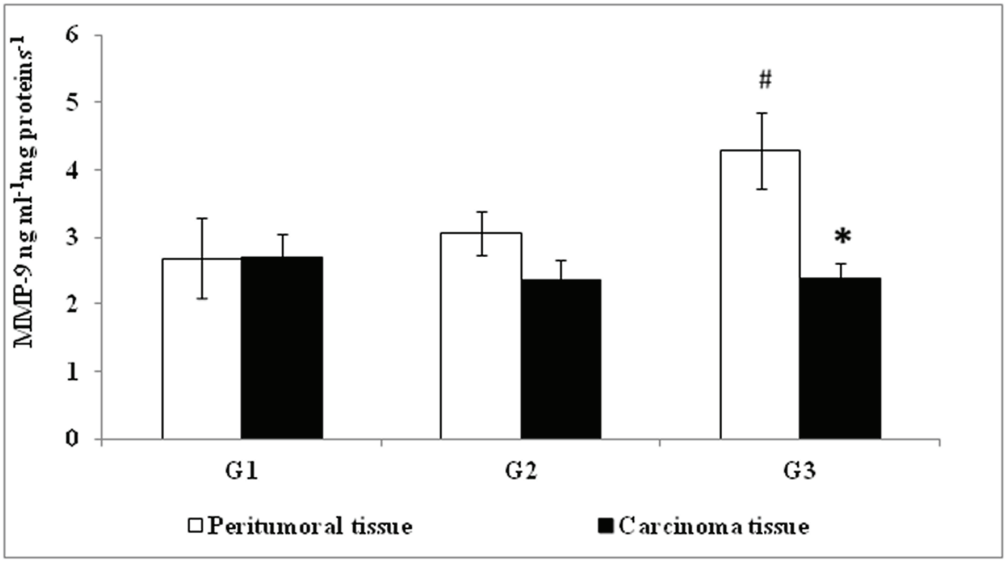

Fig. 3 – Concentration of total matrix metalloproteinase 9 (MMP-9) in peritumoral and carcinoma tissue in the breast cancer in relation to histological grade (G1 – low grade; G2 – intermediate grade; G3 – high grade).
The data were expressed as mean ± standard error (SE); n = the number of samples with G1, G2 and G3; *$p < 0.05$ statistically significant difference carcinoma *vs* peritumoral tissue; #$p < 0.05$ statistically significant difference G3 *vs* G1, G2 in peritumoral tissue.

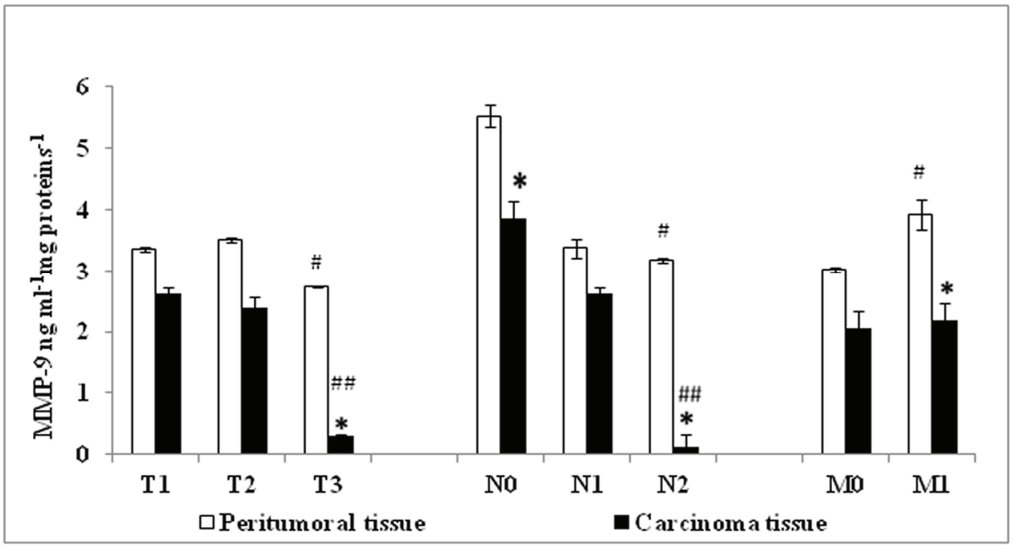

Fig. 4 – Concentration of matrix metalloproteinase 9 (MMP-9) in peritumoral and carcinoma tissue in the breast cancer in relation to tumor mode metastasis (TNM) classification.
The data were expressed as mean ± standard error (SE); n = the number of samples with T1, T2, T3, N0, N1, N2, M0 and M1; *$p < 0.05$ statistically significant difference carcinoma *vs* peritumoral tissue; #$p < 0.05$ statistically significant difference T3 *vs* T1, T2; N2 *vs* N0, N1 and M1 *vs* M0 in peritumoral tissue; ##$p < 0.05$ statistically significant difference T3 *vs* T1, T2; N2 *vs* N0, N1 and M1 *vs* M0 in carcinoma tissue.

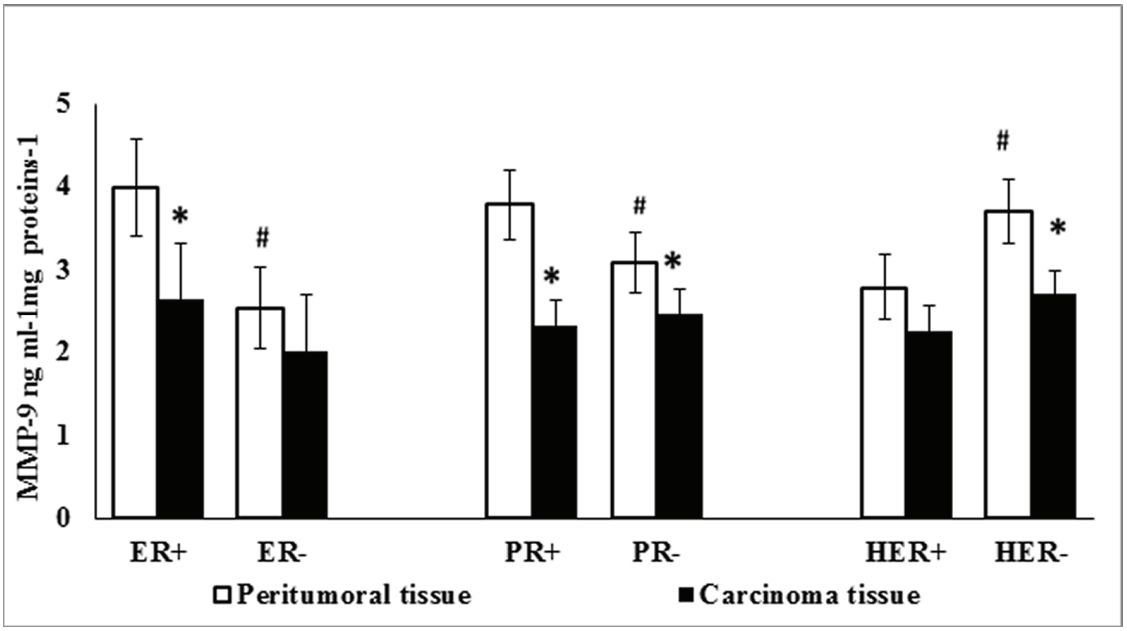

Fig. 5 – Concentration of total matrix metalloproteinase 9 (MMP-9) in peritumoral and carcinoma tissue in the breast cancer with positive (+) or negative (-) receptor expression for estrogen (ER), progesterone (PR) and human epidermal growth factor receptor (HER) [28].
The data were expressed as mean ± standard error (SE); n = the number of samples with ER +, ER-, PR +, PR-, HER + and HER-; *$p < 0.05$ statistically significant difference carcinoma *vs* peritumoral tissue; #$p < 0.05$ statistically significant difference ER + *vs* ER-; PR + *vs* PR- and HER + *vs* HER- in peritumoral tissue.

The concentration of MMP-9 was statistically higher in peritumoral tissue in the N0 and N2 groups compared to carcinoma tissue. Peritumoral tissue around the tumor with "clean" lymph nodes (N0) produced the highest concentrations of MMP-9.

There was one group of patients without confirmation of metastatic disease (Mx group). It included 12% of patients representing a limiting factor of the study. Therefore, this group was excluded from the research. When we compared the MMP-9 production in the patients with determined presence or absence of distant metastasis, we came to the conclusion that peritumoral tissue around the cancer with distant metastasis (M1) produced statistically higher concentrations of total MMP-9 compared to all other groups.

When peritumoral tissue was analyzed, it was noticed that the patient groups marked as T2, N0 and M1 produced the highest concentrations of total MMP-9, whereas the lowest concentrations were noticed in the patient groups marked as T3 (tumor size > 5 cm), N2 (metastases present in the ipsilateral axillary nodes) and M0. In carcinoma tissue, the groups T1, N0 and M1 produced the highest concentrations of MMP-9, whereas the lowest values were produced in the groups T3, N2 and M0. When the MMP-9 production ratio in peritumoral and carcinoma tissue was analyzed, it was concluded that the patients with the biggest cancer dimension (T3) had the biggest ratio 1 : 9.41. Also, there was a drastically big difference in the MMP-9 production in the N2 patient group where the ratio was 1 : 26.5, whereas in the group M1, the MMP-9 production difference between carcinoma and peritumoral tissue was 1 :1.8.

Considering that the status of receptor for estrogen, progesterone and HER2 has a significant role in the breast cancer therapy, concentrations of total MMP-9 in peritumoral and carcinoma tissue were analyzed in relation to expression of these receptors. It was proved that peritumoral tissue in all the examined patient groups produced bigger amounts of total MMP-9 compared to carcinoma tissue (Figure 5). Peritumoral tissue around cancer with the expressed steroid receptors (ER+, PR+) produced a statistically higher amount of MMP-9 compared to the environment around the cancer without these receptors. To the contrary, the concentration of total MMP-9 was higher in peritumoral tissue around the cancer with no expressed receptor for human growth factor (HER-).

In relation to the concentrations of total MMP-9 in peritumoral and carcinoma tissue in relation to patient age with diagnosed breast cancer, it can be said that the microenvironment around the tumor, in the patients younger than 40, produced statistically higher concentrations of MMP-9 compared to the same tissue in older patients.

Discussion

Since the breast cancer represents a systemic disease, surgical treatment is insufficient in treatment of these patients, so the researches on molecular and genetic level are in their full expansion. Surgical intervention is considered as properly oncologically conducted if R0 resection was performed. It means that preparation margins are "clean" both macroscopically and microscopically, without the residual

malignant cells in peritumoral tissue. The R1 resection indicates "macroscopically clean" preparation of margins, yet, the histopathological analysis indicates the presence of tumor on preparation margins. The R2 resection indicates that preparation margins are neither macroscopically nor microscopically "clean" [16, 28–31]. Our assumption is that pathohistological analysis of tumor and peritumoral tissue is insufficient and an examination on the molecular level is required for better prediction of tumor progression. Even if there are no malignant cells in peritumoral tissue, there are some changes on the level of molecular markers indicating a further course of disease. Peritumoral tissue does not represent a passive factor in pathogenesis and tumor development, so we tried to find which parameters or markers in peritumoral tissue could help us predict the progression of malignant disease. According to the literature data, considering its role in the degradation of extracellular matrix, it would be logical to expect that MMP-9 could have a significant role in these processes [32]. We consider that the determination of MMP-9 concentration is significant in prediction of biological tumor behavior in terms of disease aggressiveness not only in carcinoma but in peritumoral tissue as well. To our knowledge, there is just one study described in literature that analysed the MMP-9 concentration in peritumoral breast tissue. There was an isolated group of patients with higher risk of disease metastases and/or recidivism despite correctly conducted radical surgical R0 resection, which was indicated by the elevated level of MMP-9 in peritumoral tissue. Numerous literature data suggest that there is a correlation between MMP-9 and certain clinical and pathological parameters [21]. This study compared both MMP-9 concentration in the tumor and its environment and correlation with the clinical and pathological parameters in the patients with the breast cancer. We proved that the production of total MMP-9 was higher in peritumoral tissue than in tumor itself with a statistically significant difference. There are only a few papers with studies on the MMP-9 level in peritumoral tissue [12]. We found statistically higher concentrations of MMP-9 in peritumoral than in cancer tissue in the ductal as well as the lobular breast cancer. Results showed that the MMP-9 content in the tumor microenvironment increased with the increase of histological grade. Peritumoral tissue at G3 grade produced significantly higher concentrations of MMP-9 compared to G1 and G2 grades. The results correspond to the literature, indicating that the higher MMP-9 concentrations correlate with the less differentiated high grade cancer phenotype [32]. According to our study, there was no correlation between the MMP-9 production and the histological carcinoma type. However, both ductal and lobular types were ascertained with significantly higher concentrations in peritumoral than in carcinoma tissue. These data are in accordance with the literature data [12]. Some papers presented often contradictory attitudes about the correlation of MMP-9 serum concentration and certain clinical and pathological features, but they mostly agreed on absence of correlation between the MMP-9 level and tumor size and/or distant metastases, as well as on presence of correlation between the MMP-9 concentration and metastases in the axillar lymph nodes [33]. The results revealed that the high MMP-9 expression was associated with the small tumor size (T1) as well as with the poor differentiation (G3) which is in accordance with the literature [33]. An interesting fact is that the increase of tumor size considerably changes the concentration ratio in the tumor and peritumoral tissue. In case of T1 tumor, the ratio was 1 : 1.27 in advantage of peritumoral tissue, whereas in the tumor size T3, the ratio was even 1 : 9.41. The increase of tumor size causes the production of smaller amount of MMP-9 but peritumoral tissue continues to produce MMP-9 in unreduced amount. We consider that it is very important to analyze the MMP-9 concentrations both in peritumoral tissue and tumor since the altered ratio proves peritumoral tissue to be an active factor in tumorigenesis. Heo et al. [33] demonstrated a positive correlation between the serum and MMP-9 levels in lymph node metastasis in the breast cancer patients. In this respect, our results are consistent with the literature data. It was noticed that peritumoral tissue produced significantly more MMP-9 than carcinoma tissue in advanced disease stadium (T3, N2, M1). Such a tumor environment in developed disease takes over the role and becomes a dominant source of MMP-9, e.g., MMP-9 concentration ratio in carcinoma and peritumoral tissue was 1 : 1.44 in the N0 stadium, whereas in the N2 stadium, the ratio was 1 : 26.5. Therefore, we conclude that the MMP-9 concentration in peritumoral tissue is directly proportional to involvement degree of axillar lymph nodes. A bigger difference in favor of peritumoral tissue indicates poor prognosis. It could be concluded that even though the primary tumor is removed, MMP-9 activity will not be reduced due to the increased production in peritumoral tissue. A statistically significant difference between the concentrations of MMP-9 in peritumoral tissue and cancer tissue (in favor of the peritumoral tissue) existed only in case of metastatic disease, but not in the M0 stage. This is a very important conclusion because it highlights the importance of seeking and diagnosing still unknown metastases. The results revealed that the increased production of MMP-9 in peritumoral tissue in the breast cancer appeared in the patients with the positive ER and PR receptors [34]. Literature contains data indicating that there is no statistically significant correlation between the serum MMP-9 level on the one, and the ER, PR and HER-2 status on the other hand [35]. It was confirmed that in breast cancer estrogen could promote pathological tumor-stromal interactions through degradation and remodeling of extracellular matrix. Members of the MMP family and their inhibitors play the key role in these processes. There are evidence that ER and related compounds could participate in regulation of these processes [36–38]. We found a statistically significant difference between MMP-9 in peritumoral tissue of the PR+ patients in regard to the PR- patients, but did not demonstrate such difference in cancer tissue, respectively. The results confirmed that a higher concentration of MMP-9 was produced in the patients with unexpressed receptors for HER-2 in carcinoma and peritumoral tissue. According to the MMP-9 expression, it could be concluded which distant organs the breast cancer metastases would appear in. Therefore, in experiment with cells with the expressed HER-2 receptors and higher MMP-9

level, metastases commonly appear in the brain, whereas in the group without expressed HER-2, metastases could be expected in other organs such as bones and pleura. This suggests that analyzing MMP-9 could influence a further treatment course in these patients [39, 40]. The results showed that, in the whole group of patients, a significantly higher level of MMP-9 in peritumoral tissue was associated with the HER-2 negative breast cancer. Literary data claim that the MMP-9 overexpression in the carcinoma cells was associated with the HER-2 overexpression, but only in the subgroup of node-negative patients. Also, the stromal MMP-9 overexpression was associated with the HER-2 overexpression only in the ER+ tumors. It suggests that the relation among the MMP-9 levels and different clinical and pathological features is very complicated and further investigations are needed [32]. In all age categories, the MMP-9 concentration is higher in peritumoral tissue than in carcinoma tissue. Additionally, the MMP-9 concentration in peritumoral tissue was a statistically significantly higher in the group of patients younger than 40 years compared to those older than 40 years. This result agreed with that of Rashad et al. [41].

Conclusion

Our research confirms that breast cancer even in early stage without regional lymph or distant metastases leads to changes in peritumoral tissue detectable on the molecular but not on histopathological level. The analysis of the total MMP-9 concentration indicates that peritumoral tissue does not represent a passive factor in the process of development and dissemination of malignant disease. Tumor environment in the developed disease takes over the role becoming a dominant source of MMP-9. Peritumoral tissue at distance of 3 cm from the tumor produces more MMP-9 than the tumor itself. The increase of tumor size and involvement of axillar lymph nodes changes the ratio of MMP-9 concentration in the tumor and peritumoral tissue in favor of peritumoral tissue. In N0 stage, the concentration ratio of MMP-9 in the tumor and peritumoral tissues is 1 : 1.44, but in N2 stage ratio is 1 : 26.5. A statistically significant difference between the concentrations of MMP-9 in the peritumoral tissue and cancer tissue (in favor of peritumoral tissue) exists only in case of metastatic disease, but not in M0 stage. This is a very important conclusion because it highlights the importance of early detecting of still unknown metastases in such cases.

Conflict of interest

The authors have declared that no competing interests exist.

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