ANGIOGENESIS IN BREAST CANCER. INCREASED VASCULARITY IS RELATED TO THE EARLY STAGES OF THE DISEASE.

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Abstract. Quantification of the extent of vascularity, expressed as a "microvessel density", was proved to be an independent prognostic indicator in breast carcinoma. Aim of the following investigation was to study the microvessel density in breast cancer and noncancereus mammary tissues. We investigated fifteen cases of breast carcinoma and five cases representing normal breast tissue. Blood vessel density was determined by light microscopy according to the described by Weidner method. Correlation between tumor size and MVD of tumors was found. CD 34 appears to be useful and sensitive marker for the MVD determination in breast cancer,
key words: Breast cancer, Neovascularization, CD 34

INTRODUCTION

Many investigations carried out during the 1990s proved that quantification of the extent of vascularity, expressed as a "microvessel density", was an independent prognostic indicator in a variety of malignancies [14], including breast carcinoma [4, 11].

Some clinical studies found correlation between angiogenesis expressed as the number of microvessels within the tumor and tumor size, histological grade, and axillary node metastases[l, 15]. Other authors did not found any correlation at all [8, 9]. At present it is belived that microvessel density is not a measure of the angiogenic dependence of a tumor. It is considered that, the vascularity of a tumor to a large degree reflects the metabolic burden of the supported tumor cells [7] .

AIM

Aim of the following investigation was to study the microvessel density in breast cancer and noncancereus mammary tissues.

MATERIALS AND METHODS

Detailed chart review of 33 clinical cases (28 cases of breast carcinoma , and 5 cases representing normal breast tissue) was performed in the Department of Surgical Oncology, University Centre of Oncology, Pleven, Bulgaria. After the removal of all cases with inadequate tissue sample, tissue staining and chart data, there remained twenty cases - fifteen cases of breast carcinoma and five cases representing normal breast tissue. Classified according to tumor size (p. TNM post-surgical histopathological classification),with noninvasive (in situ) tumors divided in a separate group, there were: 3 cases in T in situ group and twelve invasive breast tumor- 4 cases in T1 group, 6 cases in T2 group and 2 cases in T3 group. The breast cancer patient group consisted of fifteen females aged 45 to 87 (mean, 59.8) years. Axillary lymph node status, Her2, estrogen and progesterone receptor status of the breast cancer patients and tumors' differentiation were also clinical variables of interest in this study. The cases of noncancerous mammary tissues consisted of 5 females aged 46 to 72 (mean, 58.2) years.

Imunohistochemical assessment of the specimens was preformed with CD34 monoclonal antibody (Dako, Glostrup, Denmark) according to protocol, at the Department of General and Clinical Pathology Pleven, Bulgaria.

Blood vessel density was determined by light microscopy according to the described by Weidner [15] method, with some minor modifications combined with computer assisted analysis. The observer identified the "hot spots" by scanning at low power (xlOO) (Leica stereo microscope, model DM100030W). Then the chosen 10 separate arias within the "hot spot" (0.0558 mm^2 each)were photographed at x400 magnification with Leika stereo microscope model DM100030W equipped with 1.3IMP camera and saved as 8 bit images on a computer hard disk for analysis or review. The pictures were then analysed by Scion Image (Scion Image for Windows, Scion Corporation, Frederick, Maryland).

Statistical analysis of the collected data was performed with STATGRAPHICS Plus (Statistical Graphics Corp. 1994-1996). Differences between means were considered significant if the p values were equal to or less than 0.05.

RESULTS

In analysis of the differences in microvascular density among all studied tumors (non- invasive- T in situ, and invasive- TI, T2 and T3 tumors) and noncancerous mammary tissues we found that the tumors in situ had vascular density which was increased compared to that of invasive tumors from all size groups and noncancerous mammary tissues. The vascular density was 615,33 ± 133,00 /0,558mm^2 in the tumors in situ in contrast to T1 272,25 ± 45,36/0,558mm^2, T2 187,33 ± 34,91/0,558mm^2 and T3 108,50 ± 13,43/0,558mm^2 invasive tumors and noncancerous mammary tissues 82,00 ± 8,74/0,558mm^2. We also found that T1 tumors 272,25 ± 45,36/0,558mm^2 had increased vascularity compared to T2 187,33 ± 34,91/0,558mm^2, T3 108,50 ± 13,43/0,558$mm^2$and noncancerous mammary tissues 82,00 ± 8,74/0,558mm^2(p=:0.00I). The T2 tumors in the same time had significantly increased vascular density compared to T3 tumors and noncancerous mammary tissues (p=0,002). Tumors larger than 5 cm (T3) had vascular density 108,50 ± 13,43/0,558mm^2 which did not differed significantly from the value of that in noncancerous mammary tissues 82,00 ± 8,74/0,558mm^2 (p>0.05)Table. 1

We failed to prove correlation between some valuable prognostic factors for breast cancer and microvascular density. Table. 2

Table 1

	№ of cases:	MVD (0.558mm2) mean ± SD
T in situ	3	615,33 + 133,00
Invasive Breast cancer T1 (<2 cm)	4	272,25 ± 45,36
T2 (2-5cm)	6	187,33 + 34,91
T3 (>5 cm)	2	108,50 ± 13,43
Noncancerous mammary tissues	5	82,00 ± 8,74

MVD- microvascular density, SD- standard deviation

DISCUSSION

When compared, data from different studies available in literature was found to be controversial. The observed differences were mainly due to different approaches used in the process of MVD assessment of breast tumors. Some authors believed that the differences in MVD values were due to subjectivism in the determination of the "hot spot" inside the tumor [2, 13]. Other authors claimed that vascular density depended on histological type and tumor size [10]. According to a study by Yamaura H and Sato H, there were changes in microvessel diameter and microvessel counts along with the stages of a tumor [17]. Those differences were also possible reason for certain variations in the results between studies. Results also strongly depend on the used endothelial cell marker[2, 5, 12].

All these considerations were taken into the process of interpretation of our results and comparing them to results from other studies.

According to our study noninvasive carcinomas had higher value of vascular density than did invasive carcinomas from all size groups and noncancereus mammary tissues. The fact that in situ tumors had significantly increased MVD compared to invasive carcinomas corresponded to data found in literature [10]. In these cases we observed many microvessels around the affected by the tumor mammary duct. This typical vascularization pattern described as: angiogenesis emerging around a breast duct containing carcinoma in situ, that developed in one or two rows of microvessels closely apposed to the duct was corespondet to data found in litrature[3]. We found that MVD of invasive breast cancer decreased progressively in association with the increase of tumor size from pTl to pT3 probably again due to change in the balance of angiogenic-antiangiogenic stimuli. Despite the significant differences in the MVD in invasive and noninvasive breast tumors as well as the difference of vascularity among different size invasive tumors, the question about the circulatory efficiency of these newly formed blood vessels remains unclear.

We have to notice that in our tumor groups there were no tumors with size ± 0, 4 cm near the T groups' margins. Since according to the p TNM classification tumors are separated in different tumor groups that have small difference in metric size and respectively close values of MVD it should be carefully used in studies concerning vascular density. For example a 1, 8 cm tumor should be evaluated as pTl and 2, 1 cm tumor as pT2 while the only 0, 3 cm difference between their metric sizes suggests close values of their MVD and no significant differences in MVD between T1 and T2 tumors (wich can be the explanation of the fact that some studies claimed no corealiono of the tumors' size with vascular dencity). None of the predictive factors in breast cancer that we studied was related to the MVD of the tumors. The MVD appeared to be an independent prognostic factor in breast cancer.

CONCLUSION

CD 34 appears to be useful and sensitive marker for the MVD determination in breast cancer. Results from studies concerning tumor vascularization should be carefully interpreted because of their dependence on different factors. We conceder that the MVD is an independent prognostic factor. The observed increase of MVD in noninvasive tumors as well as the decrease of vascularity corresponding to the progression of tumor growth shows that the MVD undergoes some dynamic changes during the development of tumors. It seems that even poorly vasculated T3 tumors could have had increased vascularity in their early invasive period. (According to literature [16], more vasculated tumors are considered to have worse prognosis, which in this case leads to an apparent paradox because the tumor is the same.) That is why a more detailed understanding of the factors connected to both survival in breast cancer patient and MVD in breast cancer is needed. A possible explanation cold turn to be the process of development of distant blood metastases and their ability to remain dormant for long periodwhich seemed to be connected to angiogenesis [6].

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Table 2

	No. Of cases:	MVD (0.558mm−2) mean ± SD	P
Lymph-node status N(-)	n=5	279,6±118,78	p=0,391 N S
N(+)	n=10	287,8±220,79	
HER2 -	n=11	318,91±209,91	P= 0,242 NS
+	n=3	184,67±58,59	
ESTROGEN receptor status -	n=6	210,00±47,87	P = 0,775 N S
+	n=7	331,29±255,25	
PROGESTERONE receptor status	n=5	199,20±67,15	P = 0,465 NS
+	n=6	357,67±255,81	
Histological grade HGI (Well)	n=3	329,00±134,10	
HGII (Moderate)	n=9	313,33±221,23	P = 0,149 NS
HGIII (Poor)	n=3	156,33± 50,84	

MVD-microvessel density, SD- standard deviation, NS- not significant, p- evaluated by one way factorial ANOVA (Kruskal - Wallis test)