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The relations of vascular endothelial growth factor–C and lymph node metastasis in breast cancer patients

Meme kanseri hastalarında vasküler endotelyal büyüme faktörü-C ve lenf nodu metastazı ilişkisi

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Abstract

Aim: Breast cancer (BC) is the 2nd most common cancer worldwide and most of the cases are sporadic. BC chooses mainly the lymphatic system and axillary lymph nodes (LN) are frequently involved. Lymph node metastasis (LNM) is driven by tumor-derived lymphangiogenic growth factors, especially vascular endothelial growth factor (VEGF) family. Higher serum levels of VEGF-C have been detected in cases of BC. In the present study, we aim to investigate the expression pattern of VEGF-C in tumor specimens and the relation of VEGF-C expression with LNM by immunohistochemistry.

Methods: In this clinical, cross-sectional study, paraffin-embedded specimens were obtained from 16 female patients who had primary invasive ductal breast cancer and received surgical treatment between April 2006 and March 2007. BC tissue sections were stained with VEGF-C antibody and evaluated according to the severity and intensity of the staining. Statistical analysis was performed using SPSS version 15.0 for Windows and in all analyses, a 'p' value less than 0.05 was considered statistically significant and comparisons were 2-tailed.

Results: The average age at the time of diagnosis was 50 years (range: 24-82 years). In the present study, on the basis of SI and SS VEGF-C expression did not show statistically significant correlation with LNM, while calculated IRS - as a variable- was correlated with LNM. The grade of the tumor correlated neither with the VEGF-C expression nor with lymph node metastasis ($p>0.05$).

Conclusions: It is interesting that the correlation with VEGF-C IRS score and axillary lymph node level was found significant statistically. In the current study, we demonstrated the VEGF-C relation with LNM via IHC staining in tumoral samples of BC patients.

Keywords: Vascular endothelial growth factor–C, Lymph node metastasis, Breast cancer

Öz

Giriş: Meme kanseri (MK) dünya çapında en sık görülen 2. kanserdir ve vakaların çoğu sporadiktir. MK yayılım için esas olarak lenfatik sistemi seçer ve aksiller lenf düğümleri (LN) sıklıkla bu yayılımda rol alır. Lenf nodu metastazı (LNM), tümör kaynaklı lenfanjiyojenik büyüme faktörleri, özellikle vasküler endotelyal büyüme faktörü (VEGF) ailesi tarafından yönlendirilir. MK vakalarında daha yüksek oranda serum VEGF-C düzeyleri tespit edilmiştir.Bu çalışmada, tümör örneklerinde VEGF-C ekspresyon paternini ve immünohistokimyasal olarak LNM ile VEGF-C ekspresyonunun ilişkisini araştırmayı amaçladık.

Yöntemler: Bu klinik çalışmada, Nisan 2006 ile Mart 2007 arasında cerrahi tedavi alan primer invaziv duktal meme kanserli 22 kadın hastadan Parafine gömülü örnekler alındı. MK doku kesitleri VEGF-C antikoru ile boyandı ve boyanma şiddeti ve yoğunluğuna göre değerlendirildi. İstatistiksel analiz, Windows için SPSS 15.0 sürümü kullanılarak gerçekleştirildi. Tüm analizlerde, 0.05'ten küçük bir 'p' değeri istatistiksel olarak anlamlı kabul edildi ve karşılaştırmalar 2 yönlü olarak yapıldı.

Bulgular: Tanı anında ortalama yaş 50 idi (dağılım: 24-82 yıl). Bu çalışmada, SI ve SS VEGF-C ekspresyonu temelinde LNM ile istatistiksel olarak anlamlı bir ilişki bulunmazken, hesaplanan IRS - değişken olarak LNM ile ilişkili gözlenmiştir. Tümör derecesi ise ne VEGF-C ekspresyonu ne de lenf nodu metastazı ile belirgin ilişkili gözlenmemiştir ($p> 0,05$).

Sonuç: VEGF-C IRS skoru ve aksiller lenf nodu düzeyi ile ilgileşiminin istatistiksel olarak anlamlı olduğu dikkati çekmektedir. Bu çalışmada, MK hastalarının tümöral örneklerinde LNM ile VEGF-C ilişkisini IHC boyaması ile gösterdik.

Anahtar kelimeler: Vasküler endotelyal büyüme faktörü –C, Lenf nodu metastazı, Meme kanseri

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Introduction

Breast cancer (BC) is the 2nd most common cancer worldwide and it is the 5th most common cause of death due to cancer in females. Its' incidence and morbidity are increasing in developed countries in spite of advances in early diagnosis and treatment modalities [1]. Most of the BC cases are sporadic and not have genetic bases. Heterogeneity and different expression level of estrogen receptor (ER), progesterone receptor and human epidermal growth factor receptor (HER-2) are characteristics of BC and it is generally classified based on immunohistochemical expression of these hormone receptors. This classification is useful not only for identification of different types of BC but also for prognosis and treatment modality assessment [2].

BC chooses mainly the lymphatic system rather than the hematologic system to spread and axillary lymph nodes (LN) are frequently involved [3]. As a surgical treatment of BC, axillary lymph node dissection (ALND) or sentinel lymph node biopsy (SLNB) is recommended by the guidelines. Axillary lymph nodes are classified according to their location which centering pectoralis minor muscle (PMM). LN positioning at the lateral border of PMM is level I, the posterior border is level II and the medial border is level III. ALND involves removal of the axillary LN from level I and II (and in case of apparent involvement, level III) and it is required for treatment and staging purposes [4].

It is clearly shown that lymphangiogenesis is the starting pace for LN metastases. Lymphangiogenesis, the formation of new lymphatic vessels, is approved to be driven by tumor-derived lymphangiogenic growth factors, especially vascular endothelial growth factor (VEGF) family [5]. The VEGF family is comprised of different isoforms which interact with different VEGF receptor (VEGFR) types. In this family mainly VEGF-C and VEGF-D, have been identified as lymphangiogenic growth factors and bind to the VEGF receptor (VEGFR)-3, that is expressed in lymphatic endothelial cells [6]. VEGF-C is needed for the initial migration of viviparous endothelial cells and in case of tumoral growth and lymphatic spread, VEGF-C expression increases. Higher serum levels of VEGF-C have been detected, especially in cases of breast and colon cancer. It is claimed that VEGF-C promotes tumoral growth and metastases not only by means of lymphangiogenesis but also via autocrine regulation. Besides that VEGF-C protects cancer cells against oxidative stress and immune response so increases cancer cell survival rate [7].

In the present study, we aim to investigate the expression pattern of VEGF-C in tumor specimens and the relations of VEGF-Cexpression with lymph node metastasis (LNM) by immunohistochemistry.

Materials and methods

Patient and specimens

In this clinical, cross-sectional study, Paraffin-embedded specimens were obtained from 22 female patients who had primary invasive ductal breast cancer and received surgical treatment between April 2006 and March 2007 at the Department of General Surgery, Fırat University Hospital, Elazıg, Turkey. The patients who had received radiotherapy or chemotherapy preoperatively were excluded from the study. The patients with a medical history of synchronous tumor involvement (primary origin was not breast tissue) or chronic obstructive pulmonary disease, which may increase VEGF levels, were also excluded from the study. The remaining patients were evaluated (n=16) for this study and the expression levels of VEGF-C was defined by immunohistochemistry.

All patients were undergone modified radical mastectomy with standard ALND. In all cases, BC tissue, noncancerous breast epithelial tissue, and ALND materials were collected and fixed for standard histological assessment and immunostaining. Before the initiation of the study, local Research Ethics Committee of Fırat University Hospital endorsement for the project was supplied.

Demographic data and clinicopathologic characteristics of the patients were recorded. The evaluated clinicopathologic parameters were tumor size (largest tumor diameter), histological type of tumor, grade of tumor, presence of lymphovascular invasion, presence of capsule invasion, and the statue of lymph node metastasis (which described with their localizations-levels-), estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2/neu). All of the BC patients received postoperative adjuvant chemotherapy and/or hormonotherapy consisting of combination chemotherapy. Follow- up and survival of these patients were not assessed in this study.

Immunohistochemistry

During the surgery, axillary lymph node dissection was performed and the levels were marked and sorted according to lymph node levels. These lymph nodes and BC tissue samples were fixed and stored in paraffin-embedded samples for routine histological examination and confirmation of the diagnosis. Later, new sections were obtained from paraffin blocks for immunohisto-chemical (IHC) examination.

Following the completion of the study period, the formalin-fixed and paraffin-embedded BC tissue sections were stained with the VEGF-C antibody (H-190: sc-9047; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for IHC examination using standard streptavidin-biotin-horseradish peroxidase (HRP) technique. Mayer's Hematoxylin was used for counterstaining the slides. The immunohistochemical staining results were interpreted by an experienced pathologist other than the first examiner.

After staining, the tissues were evaluated according to the severity and intensity of the staining. Staining strength (SS) value is described as follows;

i) If no staining has occurred, the value is '0' (Fig. 1),

ii) If it is poorly stained, the value is '1',

iii) if there is moderate staining, the value is '2',

iv) If staining is intense, the value is '3' (Figure 2).

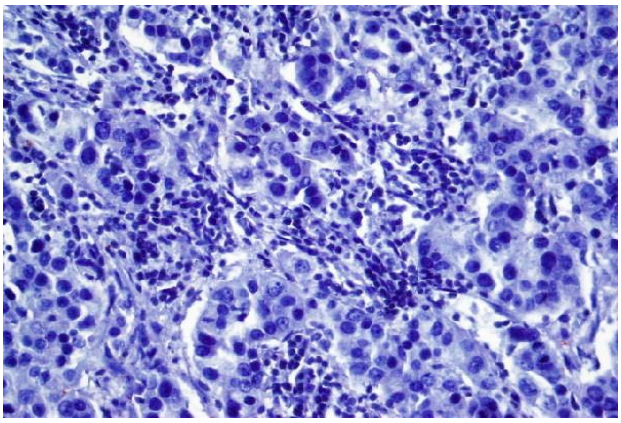

Figure 1: VEGF-C staining strength '0' (immunoperoxidase in 400 magnification)

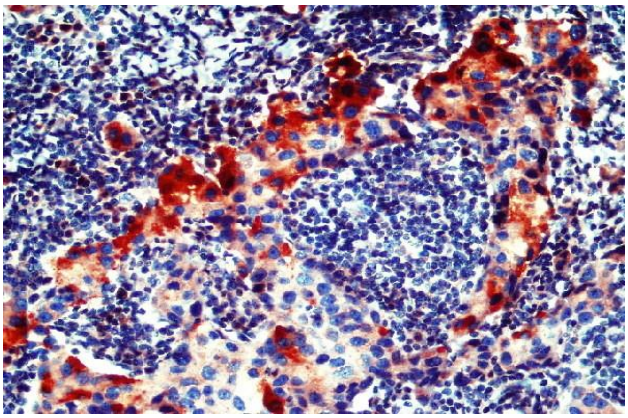

Figure 2: VEGF-C staining strength '3' (immunoperoxidase in 400 magnification)

When the staining intensity was evaluated, the ratio of the area stained positively to the entire surface of the sample was taken as the basis. According to this, staining intensity (SI);

i) If this ratio is between 0-10%, '0',

ii) If this ratio is between 10 and 25%, '1',

iii) If this ratio is between 25 and 50%,'2',

iv) If this ratio is between 50 and 75%,'3' and

v) If it is more than 75%, it is evaluated as '4'.

However, the intensity of VEGF-C staining was not considered as a parameter alone. Instead, the crude data were then transformed to an Immunoreactive Score (IRS) by adding the scores for the staining intensity and staining strength. SI, SS, IRS were considered as a variable individually.

Statistical analysis

Statistical analysis was performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL., USA). Data were analyzed, and the continuous variables were reported as mean± standard deviation (SD), and nominal variables were reported as total number and percentages.

Variables were first evaluated by One-Sample Kolmogorov-Smirnov test as a normality test to choose the type of statistical tests –parametric or non-parametric test–, and the results showed that asymp. sig. (2-tailed) levels ≤ 0.05 so we decided to use non-parametric tests. For statistical analysis, correlations between variables were evaluated for significance by using the Spearman's rho test. Categorical variables were evaluated by the Mann-Whitney U test and Kruskal Wallis t-test of contingency. In all analyses, a 'p' value less than 0.05 was considered statistically significant and comparisons were 2-tailed.

Results

All 16 patients who underwent surgery for BC during the study period were evaluated. The average age at the time of diagnosis was 50 years (range: 24-82 years). All of the patients' diagnoses were confirmed as invasive ductal cancer and the clinicopathologic properties of the patients and correlations with LN metastases were outlined in Table 1.

Table 1: Demographic data and the clinicopathologic properties of the patients and correlations with LNM*

Variable	Total cases (n=16)	Lymph node metastasis (+)(n=12)	p value+
Age	50 ±15.27	50.92 ±17.53	0.716
Grade			
I/II	6 (37.5%)	2 (16.7%)	0.03
III	10 (62.5%)	10 (83.3%)	
Size			
≤5cm	9(56.3%)	8 (66.7%)	0.159
>5cm	7(43.8%)	4 (33.3%)	
SS	1.69 ±0.94	1.83± 1.11	0.056
SI	1.56 ±1.09	1.92± 0.9	0.064
IRS	3.25 ±1.94	3.75± 1.91	0.04
ER+	3 (18.8%)	0	0.01
HER-2+	6 (37.5%)	6 (50%)	0.056

* Values are either expressed as mean±standard deviation or n (%).
+p-values calculated for comparison of survivors versus non-survivors group by Mann- Whitney U test.
Abbreviations: LNM; Lymph node metastasis, SS; Staining strength, SI; staining intensity, IRS; Immunoreactive Score, ER; estrogen receptor,HER-2; human epidermal growth factor receptor

Six patients were Grade I/ II and the majority of cases were Grade III (62.5%) and it was statistically correlated with LNM ($p<0.05$). In 7 cases, tumor size was > 5cm and this variable was not related to LNM. SI and SS were not related to LNM as a variable, whereas the correlation between LNM and calculated IRS was statistically significant ($p<0.05$). In this study, the cutoff point for the IRS was chosen as 3 (the midpoint of the IRS scale) and a new category was formed with this factor to determine the relations of immunohistochemical expression of VEGF-C and clinicopathological variables(Table 2).

Table 2: The clinicopathologic properties of the patients and correlations with IRS*

Variable	Total cases (n=16)	IRS≥3(n=10)	p value+
Age	50 ±15.27	49.3 ±17.53	0.073
Size			
≤5cm	9(56.3%)	5 (50%)	0.547
>5cm	7(43.8%)	5 (50%)	
Grade			
I/II	6 (37.5%)	3(30%)	0.439
III	10 (62.5%)	7(70%)	
SS	1.69 ±0.94	2.30 ±0.48	0.006
SI	1.56 ±1.09	2.10 ±0.99	0.006
Lymphovascular invasion	6 (37.5%)	5 (50%)	0.207
ER+	3 (18.8%)	1 (10%)	0.277
HER-2+	6 (37.5%)	6 (60%)	0.014
Capsular invasion	8 (50%)	6 (60%)	0.334
Level 0	4 (25%)	1 (10%)	
Level I	6 (37.5%)	3 (30%)	0.013
Level II	3 (18.8)	3(30%)	
Level III	3 (18.8)	3 (30%)	
Lymph node metastasis (+)	12 (75%)	9(90%)	0.082

* Values are either expressed as mean±standard deviation or n (%).
+p-values calculated for comparison of survivors versus non-survivors group by Mann- Whitney U test.
Abbreviations:SS; Staining strength, SI; staining intensity, IRS; Immunoreactive Score, ER; estrogen receptor,HER-2; human epidermal growth factor receptor

Four patients showed no axillary LNM(25%), while 6 patients had LNM in level I (37.5%), and 3 patients (18.8%) in level II and level III (skipped metastases was not observed). This variable (axillary lymph node level) and IRS factor showed a statistically significant correlation (p=0.013) whereas LNM did not show a significant correlation (p=0.082). HER-2 showed significant correlation with IRS group (p=0.014) statistically and invasion –lymphovascular or capsular invasion- had no correlation with IRS of VEGF-C. The average tumor size was determined as 4.12 cm (minimum 1-maximum 10 cm) and tumor size had no relation with LNM or IRS score.

VEGF-C expression:

In 14 cases positive reaction of tumor cells to VEGF-C staining was detected (IRS scores were 2 for 4 cases and maximum score-7 was observed in 1 case). The grade of the tumor correlated neither with the VEGF-C expression nor with lymph node metastasis ($p>0.05$).

Discussion

Currently, the most frequent malignancy in the female is the Breast cancer and LNM is reported in more than one-third of the cases. One of the most substantial prognostic determinants in BC is Lymph node statue [8]. The data about LNM is crucial to establish treatment and to predict prognosis so ALND or SLNB becomes a standard process. A complicated network of growth factors, cytokines and chemokines control lymphangiogenesis which partakes to lymphatic metastasis [9,10]. VEGF-C induced lymphangiogenesis was demonstrated by Skobe et al. [11] at first and in this study, it was proposed that overexpression of VEGF-C in BC cells vigorously increased intratumoral lymphangiogenesis and augmented metastasis to the regional lymph nodes and lungs. In the present study, VEGF-C expression in 87.5% of the cases with different IRS scores correlated with LNM. It is interesting that the correlation with VEGF-C IRS score and axillary lymph node level was found significant statistically.

Vascular endothelial growth factor-C (VEGF-C) is a well-known and highly investigated member of the VEGF family and it divvies maximum homology domain with VEGF-A, which is noted to be an important angiogenic factor, among siblings [6]. The role of VEGF-C in the lymphangiogenesis process is approved by many studies, whereas the prognostic role of VEGF-C expression for LNM in BC patients is still contentious [3,10]. Previous meta-analyses showed different results about the relation of VEGF-C with prognosis and disease-free survival in breast cancer patients [12,13]. These opposite outcomes in distinct meta-analyses cause a big dilemma and in the present study, VEFG-C is related to LNM and probably with prognosis. Unfortunately, follow-up data and information about disease-free survival were missing and no conclusion can be done about VEGF-C and prognosis relation. But LNM and prognosis relation just gives us a clue about VEGF-C effect.

In some studies, the correlation between VEGF-C expression and LNM was significant, whereas in some studies this relation was not significant [8,14]. In the present study, on the basis of SI and SS VEGF-C expression did not show statistically significant correlation with LNM, while calculated IRS - as a variable- was correlated with LNM.

One of the most aggressive molecular variants of BC is the HER-2 subtype and in the present study, it was correlated with IRS score. A similar finding was detected by Schoppmann et al. [15], overexpression of VEGF-C was related with HER-2 overexpression.

Limitations

Probably the most deeply searched factor for lymphangiogenesis and tumor lymphatic metastases is VEGF-C in the literature. Our study mainly depends on data collected previously for another unpublished study years ago, but this does not depreciate the value of the findings and results. The most important limitation of this study was the small sample size which is limiting the power of the analysis. Even so, our results are parallel to other studies showing that VEGF-C plays an important role in the lymphatic metastases of BC.

Conclusion

In the current study, we demonstrated the VEGF-C relation with LNM via IHC staining in tumoral samples of BC patients. There is a need for additional studies to explore the role of VEGF-C in the augmentation and maintenance of lymphangiogenesis in BC.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359–86. doi: 10.1002/ijc.29210.
2. Raica M, Cimpean AM, Ceausu R, Ribatti D. Lymphatic microvessel density, VEGF-C, and VEGFR-3 expression in different molecular types of breast cancer. Anticancer Res. 2011;31:1757–64.
3. Wu QW, She HQ, Liang J, et al. Expression and clinical significance of extracellular matrix protein 1 and vascular endothelial growth factor-C in lymphatic metastasis of human breast cancer. BMC Cancer. 2012;12:47.
4. Zhang B-N, Cao X-C, Chen J-Y, et al. Guidelines on the diagnosis and treatment of breast cancer (2011 edition).Gland Surgery. 2012;1(1):39-61. doi:10.3978/j.issn.2227-684X.2012.04.07.
5. Zhang Y, Liu J, Lin J, et al. The transcription factor GATA1 and the histone methyltransferase SET7 interact to promote VEGF-mediated angiogenesis and tumor growth and predict clinical outcome of breast cancer. Oncotarget. 2016;7:9859–75. https://doi.org/10.18632/oncotarget.7126.
6. Wang CA, Tsai SJ. The non-canonical role of vascular endothelial growth factor-C axis in cancer progression. Exp Biol Med (Maywood). 2015;240(6):718–24. doi:10.1177/1535370215583802
7. Wang CA, Harrell JC, Iwanaga R, et al. Vascular endothelial growth factor C promotes breast cancer progression via a novel antioxidant mechanism that involves regulation of superoxide dismutase 3. Breast Cancer Res. 2014;16:462. doi: 10.1186/s13058-014-0462-2.
8. Zhao Y-C, Ni X-J, Li Y, et al. Peritumoral lymphangiogenesis induced by vascular endothelial growth factor C and D promotes lymph node metastasis in breast cancer patients. World Journal of Surgical Oncology. 2012;10:165. doi:10.1186/1477-7819-10-165.
9. Fakhrejahani E, Toi M. Antiangiogenesis therapy for breast cancer: an update and perspectives from clinical trials. Jpn J Clin Oncol. 2014;44(3):197–207. doi:10.1093/jjco/hyt201
10. Liang B, Li Y. Prognostic Significance of VEGF-C Expression in Patients with Breast Cancer: A Meta-Analysis. Iranian Journal of Public Health. 2014;43(2):128-35.
11. Skobe M, Hawighorst T, Jackson DG, et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med. 2001;7:192–8. doi: 10.1038/84643.
12. Nakamura Y, Yasuoka H, Tsujimoto M, et al. Clinicopathological significance of vascular endothelial growth factor-C in breast carcinoma with long-term follow-up. Mod Pathol. 2003;16(4):309-14.
13. Zhang XH, Huang DP, Guo GL, et al. Coexpression of VEGF-C and COX-2 and its association with lymphangiogenesis in human breast cancer. BMC Cancer. 2008;8:4.
14. Zhang Z, Luo G, Tang H, et al. Prognostic significance of high VEGF-C expression for patients with breast cancer: An update meta analysis. PLoS One. 2016;11:e0165725. doi: 10.1371/journal.pone.0165725.
15. Schoppmann SF, Tamandl D, Roberts L, et al. HER2/neu expression correlates with vascular endothelial growth factor-C and lymphangiogenesis in lymph node-positive breast cancer. Ann Oncol. 2010;21:955–60.