CPQ Cancer (2019) 1:6
Research Article

Alterations in Serum VEGF Levels After Endocrine Therapy in Breast Cancer

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Received: 03 August 2019

Published: 27 August 2019

Keywords: *Breast Cancer; VEGF; Angioge$nesis; Tamoxifen; Herceptin, Letrozole*

Abstract

Purpose

Angiogenesis has been suggested as a potential target for anticancer treatment, either by blocking of angiogenic factors production or by blocking of endothelial cell proliferation. The effect of preoperative chemoendocrine therapy is indefinite in the regulation of angiogenic factors, but reports propose that several anticancer drugs have antiangiogenic activity.

Methods

The level of an important angiogenic factor, VEGF, was assessed in 62 breast cancer patients and 14 healthy women using ELISA method.

Results

It was observed that the concentration of serum VEGF in the patients with breast cancer was higher than that in normal subjects (P= 0.0003). Also, results show that treatment with Herceptin (P= 0.88) and Letrozole (P= 0.25) cause a decrease in the levels of VEGF in serum; though the difference between VEGF levels in BC patients treated with Tamoxifen and healthy normal controls was significant (P= 0.03).

Conclusion

Treatment of breast cancer patients with common endocrine drugs indicated that Herceptin and Letrozole decrease the concentration of serum VEGF.

Introduction

For women, the most commonly diagnosed cancer is breast cancer, which alone is expected to account for 29% all new cancer diagnoses of women in the United States. Breast cancer, BC, is the second most common causes of cancer death in women, 14% of all deaths among the United States women. The probability of developing invasive breast cancer among women from birth to death in the United States (2010 to 2012) is about 12.3% (one out of every eight women) [1].

Experimental studies propose that tumor progression and metastasis in BC are angiogenic dependent [2-4]. Solid tumors require nutrients, oxygen and the ability to evacuate waste products; and therefore neovascularization is vital to growing beyond 1~3mm [5,6]. Angiogenesis, probably, leads transformation from mammary hyperplasia to malignancy [7,8].

A number of pro-angiogenic factors and also naturally occurring inhibitors of angiogenesis have been recognized [9]. Vascular endothelial growth factor (VEGF), as an angiogenesis element, is a selective and specific mitogen factor for vascular endothelial cells [10].

Angiogenesis in the female reproductive tract can be modulates by estrogen generally by effects on endothelial cells [11]. Tamoxifen, the commonly used endocrine therapy for breast cancer, is a partial agonist for the receptor of estrogen. Some studies declared that expression of VEGF mRNA increased in breast cancer cells treated by Tamoxifen [12,13]. Therefore, there is contradicting about efficacy of Tamoxifen as an adjuvant medication for estrogen-dependent BC.

Letrozole, potent third generation aromatase inhibitor, has been revealed to be superior to Tamoxifen in terms of response rates (40% - 60% according to randomized clinical trials) and time to disease progression in postmenopausal patients as the first-line treatment for locally advanced and metastatic breast cancer [14-16].

Human epidermal growth factor receptor 2 (HER2) amplification and overexpression have key roles in the breast cancer initiation, progression and metastasis. In addition, HER2 signaling up-regulates the expression

of VEGF. Herceptin (trastuzumab) is a humanized monoclonal antibody that binds to the extracellular domain of the HER2. Herceptin has been used as a targeted therapy for HER2-overexpressed metastatic BC [17-19].

The aim of this study was to analyze the levels of VEGF in the serum of patients with different stages of breast cancer and correlation with anticancer drugs.

Material and Methods

After obtaining informed consent, a total of fourteen serum samples from healthy control subjects and sixty-two serum samples from patients with different stages of BC were collected. Samples were age-matched between the two groups, and the patients age ranged between 25 and 65 years. None of the patients suffered from known earlier diagnosed tumors, diabetes mellitus or infections. The serum samples were stored at -70°C until use.

Analysis of the VEGFR Concentration by ELISA

The VEGF concentration in serum was measured using a sensitive two-site enzyme-linked immunosorbent assay (ELISA). Serum levels of VEGF were measured by using the human VEGF ELISA kit from Abcam (Cambridge, UK) according to manufacturer's instruction.

Microtiter plates (Abcam, Cambridge, UK) were first coated with 80ng of primary anti-VEGFR antibody per well in 0.1M Tris buffer and incubated overnight. Then, the plates were blocked by EIA buffer (50mM Tris, pH 7.5, 0.3M NaCl, 0.1% Triton X-100, 1% gelatin and 1% BSA). The samples and standards were sited in triplicate and incubated for about 16 hours at room temperature. After washing the wells, a biotinylated secondary antibody was added to all wells, and incubation was accomplished overnight at room temperature. β-Galactosidase combined to avidin was added and washed two hours later. To end with, 200μM 4-methylumbelliferyl-β-galactoside (Sigma, UK) in 10mM MgCl2 buffer and 50mM sodium phosphate were added. The amount of fluorescence was measured by ELISA reader, after 40 minutes' incubation at 37°C

Statistical Analysis

The statistical analysis was performed using a one-way ANOVA to test for the significance of differences among the groups, and only values of $P \leq 0.05$ were considered to be significant.

Results

Clinical and demographic characteristics of the breast cancer patients are shown in table 1. Briefly, 59.6% of the patients had invasive ductal carcinoma, 6.4% of them had invasive lobular carcinoma, and 1.2% had ductal carcinoma in situ. Referring to available immunohistochemical data, of the 62 breast cancer patients, 71% of the patients were ER-positive and 29% were ER-negative. About 72% of patients detected with positive PR. The rate of other immunohistochemical markers comprising Ki67+, P53+, and HER2+ was 94.5, 60, and 40%, respectively (table 1).

Table 1: *Demographic and clinical characteristics of the studied population.*

Variables	Patient, n (%)	Variables	Patient, n (%)
Age		**Histological type**	
25-35	8 (12.9)	In situ	21 (33.87)
36-45	21 (33.87)	Invasive ductal carcinoma	37 (59.68)
46-55	17 (27.42)	Invasive lobular carcinoma	4 (6.45)
56-65	11 (17.74)	**Estrogen receptor**	
>65	5 (8.06)	Positive	18 (28.33)
Mean age standard deviation (range), years	49 ± 10 (27-79)	Negative	44 (70.96)
Grade		**Progesterone receptor**	
I	8 (12.9)	Positive	17 (27.42)
II	35 (56.45)	Negative	45 (72.58)
III	8 (12.9)	**HER2**	
IV	9 (14.52)	Positive	25 (40.32)
ND*	2 (3.22)	Negative	37 (59.68)
Metastasis		**P53**	
Positive	22 (35.48)	Positive	37 (59.68)
Negative	40 (64.52)	Negative	25 (40.32)
Lymph node status		**Ki67**	
Positive	32 (51.61)	Positive	3 (4.84)
Negative	16 (25.8)	Negative	59 (94.16)
ND	14 (22.58)		

*ND; Not Determined

Using ELISA assay, it was observed that the concentration of serum VEGF in the BC patients was higher than that in normal subjects. (P-value= 0.0003) Figure 1 depict distribution of VEGF levels among normal cases and breast cancer patients. Also, we studied the concentration of VEGF in the serum of the patients treated with three common hormonal therapy medications, Tamoxifen, Letrozole and Herceptin. The analysis showed that there was the significant difference in VEGF levels in patients treated with Tamoxifen in comparison to healthy normal controls (P-value= 0.03); although, there were not significant differences between VEGF levels in patients who received Herceptin (P-value= 0.88) or Letrozole (P-value= 0.25) compared to the healthy normal group (Figure 2).

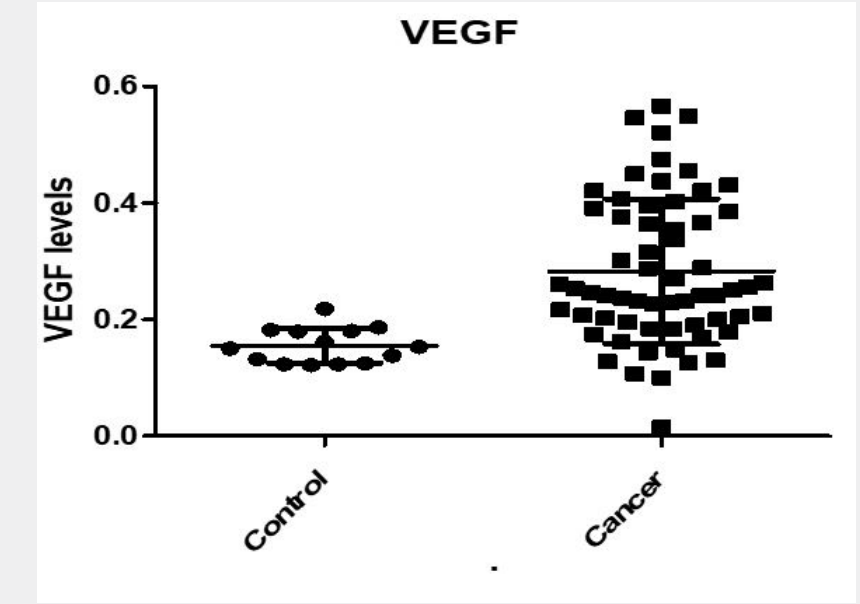

Figure 1: *Distribution of VEGF levels among normal cases and breast cancer patients*

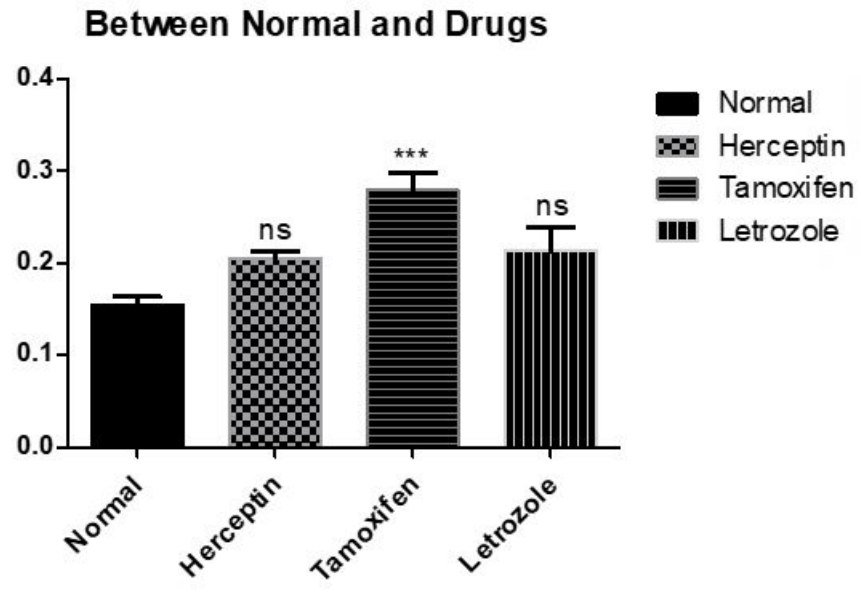

Figure 2: *The comparison of VEGF levels among breast cancer patients treated by Herceptin, Tamoxifen, Letrozole and normal persons*

Discussion

Human breast cancer represents a heterogeneous group of tumors that are diverse in behavior, outcome and response to therapy. The management of BC has involved the usage of targeted therapies even before the targets were known [20,21].

Our results showed a significant increase in serum VEGF levels in breast cancer patient's compared to healthy control group. Also, there was not a significant difference in VEGF levels of patients treated with Herceptin or Letrozole compared to healthy normal controls.

Angiogenesis is crucial that contribute to BC growth and metastasis. Vascular endothelial growth factor has been recognized as the major angiogenic factor in different cancers. VEGF increases vascular permeability and also, stimulates angiogenesis and invasion [8]. Foekens *et al.* indicated reduced survival times in cases with elevated levels of VEGF in the primary tumour, particularly in large tumors and metastatic disease [22]. Similarly, Yamamoto *et al.* found high serum and plasma VEGF levels in BC cases with larger tumours and metastatic lesion [23].

In different studies, overexpression of VEGF was showed with obvious tumor growth and neovascularization. Studies in animal models revealed that therapeutic blockage of VEGF, to inhibit the growth of the primary and metastatic tumour [9,24].

Anti-angiogenic effect of Tamoxifen on BC has been revealed, but the mechanisms of this effect have not been fully understood [25]. Garvins *et al.* (2005) [25] indicated in breast cancer cells *in vitro* that oestradiol reduced extracellular sVEGFR-1 and elevated extracellular VEGF; adding Tamoxifen reversed these effects, significantly. Hence, they suggested that the anti-angiogenic effect of Tamoxifen could be explained, in part, by its effects on VEGF and sVEGFR-1 [25].

Adams *et al.* (2000) [26] show that VEGF expression was significantly correlated with estrogen receptor status and correlated with tumour grade, inversely; also, they declared that Tamoxifen-induced increases in VEGF may be important in clinical prognosis or associated pathologies [26]. Our results, in contrast, showed BC patients who treated with Tamoxifen had a significant difference in VEGF levels in comparison with healthy controls, it means Tamoxifen has no significant effect on down-regulation of VEGF.

Letrozole has been commonly recommended as an adjuvant therapy for postmenopausal BC patients; though some hormone-dependent BC patients do not respond to Letrozole [27,28]. It has been shown that Letrozole, as a preoperative therapy, in postmenopausal patients with ER+ and/or PgR+ primary untreated BC is more effective than Tamoxifen [14]. Mary O'Neill *et al.*, *in vitro*, showed that Letrozole, unlike Tamoxifen, enhances the cytotoxicity of both doxorubicin and docetaxel; therefore it seems to be useful prescribed Letrozole with chemotherapy in ER-positive BC post-menopausal patients [29]. Our data indicated that BC patients who treated with Letrozole showed no significant difference in VEGF levels in comparison with healthy controls, in the other words Letrozole could decrease the VEGF levels in BC patients.

VEGF levels have associated with HER2 expression [30]. Expression of VEGF is regulated by HER2 signaling [31]. Forced expression of HER2 can cause up-regulation of VEGF, whereas inhibition of HER2 signaling, through down-regulating the PI3K activity with Herceptin or HER2 siRNA, decreases the VEGF levels [32]. Hui Guan *et al* (2005) [33] showed that Herceptin, an anti-cancer agent that target HER-2/neu, inhibits both HER-2/neu and VEGF expression in Ewing's sarcoma cells and enhances their sensitivity to taxol [33]. We showed that Herceptin treated patients showed no significant difference in VEGF levels in comparison with healthy controls, in the other words Herceptin could decrease the VEGF levels in BC patients.

Conclusion

In conclusion, our findings showed that Letrozole and Herceptin are potent anti-cancer drugs in down-regulation of vascular endothelial growth factor (VEGF).

Bibliography

1. Siegel, R. L., *et al.* (2016). Cancer statistics, 2016. *CA: a Cancer Journal for Clinicians, 66*(1), 7-30.

2. Brem, S. S., *et al.* (1977). Angiogenesis: a marker for neoplastic transformation of mammary papillary hyperplasia. *Science, 195*(4281), 880-882.

3. Weinstat-Saslow, D. L., *et al.* (1994). Transfection of thrombospondin 1 complementary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. *Cancer Research, 54*(24), 6504-6511.

4. Zajchowski, D. A., *et al.* (1990). Suppression of tumor-forming ability and related traits in MCF-7 human breast cancer cells by fusion with immortal mammary epithelial cells. *Proceedings of the National Academy of Sciences, 87*(6), 2314-2318.

5. Folkman, J., *et al.* (1966). Tumor behavior in isolated perfused organs: *in vitro* growth and metastases of biopsy material in rabbit thyroid and canine intestinal segment. *Annals of Surgery, 164*(3), 491.

6. Gimbrone, M. A., *et al.* (1974). Tumor growth and neovascularization: an experimental model using the rabbit cornea. *Journal of the National Cancer Institute, 52*(2), 413-427.

7. GUINEBRETIÈRE, J. M., *et al.* (1994). Angiogenesis and risk of breast cancer in women with fibrocystic disease. *Journal of the National Cancer Institute, 86*(8), 635-636.

8. Sa-nguanraksa, D. (2012). The role of vascular endothelial growth factor a polymorphisms in breast cancer. *International Journal of Molecular Sciences, 13*(11), 14845-14864.

9. Gasparini, G. (2000). Prognostic value of vascular endothelial growth factor in breast cancer. *The Oncologist, 5*(Supplement 1), 37-44.

10. Ferrara, N. & Henzel, W. J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochemical and Biophysical Research Communications, 161*(2), 851-858.

11. Losordo, D. W. & Isner, J. M. (2001). Estrogen and angiogenesis a review. *Arteriosclerosis, Thrombosis, and Vascular Biology, 21*(1), 6-12.

12. Garvin, S. & Dabrosin, C. (2003). Tamoxifen inhibits secretion of vascular endothelial growth factor in breast cancer *in vivo. Cancer Research, 63*(24), 8742-8748.

13. Ruohola, J. K., *et al.* (1999). Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells. *Molecular and Cellular Endocrinology, 149*(1-2), 29-40.

14. Eiermann, W., *et al.* (2001). Preoperative treatment of postmenopausal breast cancer patients with letrozole: a randomized double-blind multicenter study. *Annals of Oncology, 12*(11), 1527-1532.

15. Mouridsen, H., *et al.* (2001). Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III study of the International Letrozole Breast Cancer Group. *Journal of Clinical Oncology, 19*(10), 2596-2606.

16. Smith, I. E., *et al.* (2005). Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. *Journal of Clinical Oncology, 2*3(22), 5108-5116.

17. Burstein, H. J. (2005). The distinctive nature of HER2-positive breast cancers. *New England Journal of Medicine, 353*(16), 1652-1654.

18. Singh, R., *et al.* (2013). Combined blockade of HER2 and VEGF exerts greater growth inhibition of HER2-overexpressing gastric cancer xenografts than individual blockade. *Experimental & Molecular Medicine, 45*(11), e52.

19. Wen, X., *et al.* (2006). HER2 signaling modulates the equilibrium between pro-and antiangiogenic factors via distinct pathways: implications for HER2-targeted antibody therapy. *Oncogene, 25*(52), 6986-6996.

20. Dent, S. (2009). The role of VEGF in triple-negative breast cancer: where do we go from here? *Annals of Oncology, 20*(10), 1615-1617.

21. Rakha, E. A., *et al.* (2007). Prognostic markers in triple-negative breast cancer. *Cancer, 109*(1), 25-32.

22. Foekens, J. A., *et al.* (2001). High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. *Cancer Research, 61*(14), 5407-5414.

23. Yamamoto, Y., *et al.* (1996). Concentrations of vascular endothelial growth factor in the sera of normal controls and cancer patients. *Clinical Cancer Research, 2*(5), 821-826.

24. Benjamin, L. E. & Keshet, E. (1997). Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. *Proceedings of the National Academy of Sciences, 94*(16), 8761-8766.

25. Garvin, S., *et al.* (2005). Effects of oestradiol and tamoxifen on VEGF, soluble VEGFR-1, and VEGFR-2 in breast cancer and endothelial cells. *British Journal of Cancer, 93*(9), 1005-1010.

26. Adams, J., *et al.* (2000). Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen. *Cancer Research, 60*(11), 2898-2905.

27. Group, B. I. G. C. (2005). A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *N Engl J Med., 353*(26), 2747-2757.

28. Liu, Y., *et al.* (2014). Everolimus in combination with letrozole inhibit human breast cancer MCF-7/Aro stem cells via PI3K/mTOR pathway: an experimental study. *Tumor Biology, 35*(2), 1275-1286.

29. O'Neill, M., *et al.* (2012). The aromatase inhibitor letrozole enhances the effect of doxorubicin and docetaxel in an MCF7 cell line model. *BioDiscovery, 6*.

30. Yang, W., *et al.* (2002). ErbB2 overexpression correlates with increased expression of vascular endothelial growth factors A, C, and D in human breast carcinoma. *Cancer, 94*(11), 2855-2861.

31. Klos, K. S., *et al.* (2006). ErbB2 increases vascular endothelial growth factor protein synthesis via activation of mammalian target of rapamycin/p70S6K leading to increased angiogenesis and spontaneous metastasis of human breast cancer cells. *Cancer Research, 66*(4), 2028-2037.

32. Le, X. F., *et al.* (2008). Specific blockade of VEGF and HER2 pathways results in greater growth inhibition of breast cancer xenografts that overexpress HER2. *Cell Cycle, 7*(23), 3747-3758.

33. Guan, H., *et al.* (2005). Herceptin down-regulates HER-2/neu and vascular endothelial growth factor expression and enhances taxol-induced cytotoxicity of human Ewing's sarcoma cells *in vitro* and *in vivo*. *Clinical Cancer Research, 11*(5), 2008-2017.