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Association of VEGF with Regional Lymph Node Metastasis in Breast IDC

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Vascular Endothelial Growth Factor (VEGF) is a known contributor to angiogenesis in tumor growth. Its functions are numerous and include up-regulation of anti-apoptotic factors, vascular endothelial cell proliferation and migration and enhanced vascular permeability. This study aimed at evaluating association of VEGF expression with regional (axillary) lymph node metastasis in patients with breast Invasive Ductal Carcinoma (IDC). Paraffin-embedded specimens obtained from 80 female patients with breast IDC were immunohistochemically assessed in Imam Reza Teaching Centre in a 12 month period of time. These specimens were categorized into two groups based on presence or absence of axillary lymph node metastasis (n = 40 for each group). Status of the VEGF was compared between the two groups. Both groups were comparable for age and cancer laterality. Mean tumor size, as well as the percentage of cases with grade III cancer was significantly higher in the group with axillary metastasis. "Rich" expression of VEGF was documented in 40% of the cases with nodal involvement vs. 25% of the cases with nodal spare. There was no significant difference between the two groups with regard to the status of VEGF expression (p = 0.15). This difference was again insignificant after adjusting for tumor size and grade. In conclusion, there is apparently no significant association between severity of VEGF expression and axillary lymph node metastasis in patients with breast IDC.

Key words: Axillary lymph node, metastasis, breast carcinoma, vascular endothelial growth factor, invasive ductal carcinoma, childhood arthritis, rheumatoid epidemiology

INTRODUCTION

Breast cancer is the most prevalent malignant tumor among females and the first cause of death due to cancer in this group. It is estimated that about 2 million new cases of breast cancer occur each year all over the world (Abbasi *et al.*, 2009; Makarian *et al.*, 2007; Hashemi and Karami-Tehrani, 2006; Xu *et al.*, 2011). One of the main subtypes of this malignant condition is invasive ductal carcinoma (IDC) (Madigan *et al.*, 1995).

Angiogenesis is believed to play a very important role in development of vascular supply in various diseases including neoplastic and malignant conditions (ELhelaly *et al.*, 2009). Like other tumors, growth and metastasis of breast cancer is dependent on angiogenesis. Vascular Endothelial Growth Factor (VEGF), on the other hand, is a known and potent contributor to angiogenesis in breast cancer (Rafi *et al.*, 2011; Zhu *et al.*, 2006; Haghjooyjavanmard *et al.*, 2009).

VEGF which is also known as Vasculotropin and Vascular Permeability Factor (VPF), is a specific glycoprotein with molecular weight of 34-35 kD. This factor acts through stimulation of endothelial cell proliferation, inducing cell differentiation, enhancing vascular leakage and mediating vasodilatation via increasing endothelial nitric oxide syntheses within endothelial cells. Moreover, it prevents apoptosis by alteration of agents involve in matrix remodeling such as plasminogen activator inhibitor-1, plasminogen activator and interstitial collagenase and so elongates vascular survival time (Martin and Weber, 2000; Rosai, 2004). VEGF is predominantly produced by tumoral cells; however, it is shown that stromal cells may also play a rule in this regard (El-Habashy *et al.*, 2006).

It is well-known that the status of axillary lymph nodes in terms of presence and absence of metastasis is a major prognostic factor in patients with breast cancer (Greenlee *et al.*, 2000). It is not known, however, if there is an association between expression of VEGF and involvement of axillary lymph nodes in cases with breast IDC. This study aimed at investigating possible association between the status of VEGF and local metastasis in breast IDC.

MATERIALS AND METHODS

Study design and subjects: In a cross-sectional study, 80 female patients with breast IDC were recruited from Tabriz Imam Reza Educational Centre in a 12-month period of time from May 2010 to May 2011. These patients were allocated into two groups: with (n = 40) and without axillary lymph node metastasis (n = 40). The status of

VEGF expression was determined and compared between the two groups.

This study was approved by the Ethics Committee of Tabriz University of Medical Sciences and informed consents were obtained from the participants at the beginning of study.

Procedures: Presence or absence of axillary lymph node metastasis was determined by an experienced oncopathologist by evaluating properly prepared dissected specimens. The tumor grading was made according to the Nottingham Modification of the Bloom-Richardson System (Rosai, 2004). The tumor size was determined macroscopically.

Paraffin-embedded specimens obtained from tumor were immunohistochemically processed (EnVision[®], Monoclonal Mouse Anti-Human VEGF, Dakocytomation[®], Denmark). Status of stained cytoplasmic VEGF was determined by a skilled pathologist and reported as “poor” or “rich” (Fig. 1) (Turley *et al.*, 1998). This pathologist was unaware of the patients’ group.

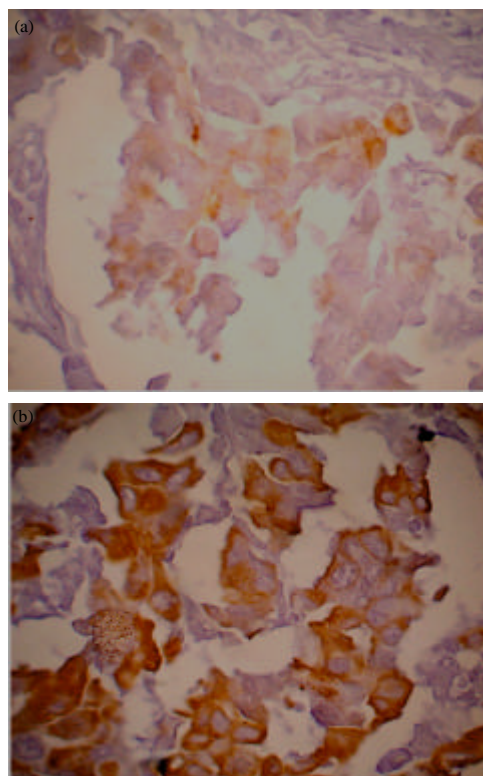


Fig. 1(a-b): Status of vascular endothelial growth factor expression in breast cancer: (a) Poor vs. (b) Rich expression reported by oncopathologist

Variables: Status of VEGF expression was compared between the two groups with and without axillary lymph node metastasis. Other studied variables were the patients' age and tumor laterality, size and grade.

Statistical analysis: Statistical evaluation was made using SPSS for Windows V 18.0 (SPSS Inc., IL, USA). Data were shown as frequency (percentage) or Mean±Standard Deviation (SD). Independent Samples t-test, χ^2 or Fisher's exact tests were employed for comparison. The p-values less than 0.05 were regarded as significant.

RESULTS

The two studied groups including patients with and without axillary lymph node metastasis were comparable for the patients' age, as well as the tumor laterality. Mean tumor size, however, was significantly higher in the group with metastasis in the axillary lymph nodes (4.70±2.05 vs. 3.34±1.58 cm; p = 0.003). Furthermore, percentage of cases with grade III breast cancer was significantly higher in the group with lymph node metastasis (25 vs. 10%, p = 0.03) (Table 1).

VEGF expression was reported to be poor and rich in 24 and 16 cases in the group with lymph node metastasis, respectively. The corresponding rates were 30 and 10 cases in the group without lymph node metastasis, respectively (Fig. 2). Accordingly, there was no significant difference between the two groups in terms of the status of VEGF expression (p = 0.15).

Table 1: Characteristics and general data of patients with and without lymph node involvement

Variable	With meta stasis (n = 40)	Without meta stasis (n = 40)	p-value
Age (year)	49.35±11.01 (27-74)	50.10±11.16 (32-88)	0.760
Side			
Right	16 (40)	21 (52.5)	0.260
Left	24 (60)	19 (47.5)	
Tumor size (cm)	4.70±2.05 (2-10)	3.34±1.58 (1-7)	0.003
Grade			
I: ≤2 cm	7 (17.5)	17 (42.5)	0.030
II: 2-5 cm	23 (57.5)	19 (47.5)	
III: 5 cm	10 (25)	4 (10)	

Values are Mean±standard deviation (range) or frequency and (%) p<0.05 is considered statistically significant

Table 2: Status of the VEGF expression in patients with and without lymph node metastasis with regard to tumor size

Tumor size	VEGF status	Poor	Rich	p-value
I: ≤2 cm	With metastasis	3 (75.0)	1 (25.0)	0.99
	Without metastasis	5 (55.6)	4 (44.4)	
II: 2-5 cm	With metastasis	17 (70.8)	7 (29.2)	0.41
	Without metastasis	21 (80.8)	5 (19.2)	
III: >5 cm	With metastasis	4 (33.3)	8 (66.7)	0.13
	Without metastasis	4 (80.0)	1 (20.0)	

Values are frequency and (%), p<0.05 is considered statistically significant, VEGF: Vascular endothelial growth factor

Status of VEGF expression is compared between two groups with and without axillary lymph node metastasis in Table 2, stratified by tumor size. In none of the tumor size sub-groups (≤2, 2-5, >5 cm), a significant difference was detected between the two groups with and without metastasis in terms of poor or rich VEGF expression (Table 2).

Frequency of poor and rich VEGF expressions is compared between patients with and without regional metastasis in Table 3, stratified by tumor grade. In different subgroups of patients with various tumor grades (I: ≤2 cm, II: 2-5 cm and III: 5 cm) no significant difference was detected between the patients with and without axillary lymph node metastasis with regard to status of VEGF expression (Table 3).

DISCUSSION

In the current study status of VEGF expression was assessed and compared in patients with breast IDC with and without axillary lymph node invasion. Although frequency of the rich expression of VEGF was higher in the patients with nodal metastasis, this difference was not statistically significant (40 vs. 25%; p = 0.15). This difference was not again significant after adjustment for tumor size and grade. To the best of our knowledge, there is not any similar study in the literature concentrating on association of the VEGF expression and axillary lymph

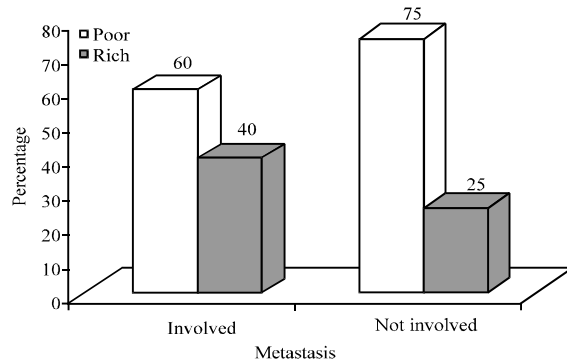


Fig. 2: Status of vascular endothelial growth factor expression in breast cancer patients with and without axillary lymph node metastasis

Table 3: Status of the VEGF expression in patients with and without lymph node metastasis with regard to tumor grade

Tumor size	VEGF status	Poor	Rich	p-value
I: ≤2 cm	With metastasis	3 (42.9)	4 (57.1)	0.17
	Without metastasis	13 (76.5)	4 (23.5)	
II: 2-5 cm	With metastasis	16 (69.6)	7 (30.4)	0.77
	Without metastasis	14 (73.7)	5 (26.3)	
III: >5 cm	With metastasis	5 (50.0)	5 (50.0)	0.58
	Without metastasis	3 (75.0)	1 (25.0)	

Values are frequency and (%), p<0.05 is considered statistically significant, VEGF: Vascular endothelial growth factor

nod metastasis. However, even the available data are not conclusive. In line with our report, many a number of investigations have concluded that there is not a prognostic role for VEGF expression in breast cancer. Lymph node involvement has been a part of evaluation in these studies (Anan *et al.*, 1998; Hoar *et al.*, 2003; Ludovini *et al.*, 2003; Toi *et al.*, 1995; Kinoshita *et al.*, 2001; Yang *et al.*, 2002).

In contrast, however, some other studies believe that expression of VEGF in breast cancer may be associated with poorer prognosis in patients with breast cancer. Lymph node metastasis as an indicator of more advanced malignancy has been shown to be associated with higher levels of the VEGF expression, as well (Valkovic *et al.*, 2002; Gasparini *et al.*, 1997; Linderholm *et al.*, 1998, 2000).

Apparently the latter group does not confirm our results in this regard. Nevertheless it should be noticed that the rate of cases with rich expression of VEGF was higher in patients with axillary nodal metastasis with no significant difference. Many factors may be discussed in justification of this heterogeneity in the literature. There is variety of VEGF subtypes recognized by now. It is assumed that only some particular subtypes (such as A, C and D) may be associated with angiogenesis and lymphangiogenesis in breast cancer (Skobe *et al.*, 2001; Nakamura *et al.*, 2005; Lee *et al.*, 2002; Mattila *et al.*, 2002; Al-Rawi *et al.*, 2005; Choi *et al.*, 2005).

In the present study we did not determine these subtypes. Further studies focusing on this issue may be helpful. Some studies believe that the VEGF level of the cancerous tissue is more important than in serum (Van den Eynden *et al.*, 2007; Anan *et al.*, 1996; Yoshiji *et al.*, 1996; Schoppmann *et al.*, 2006).

So, simultaneous assessment of VEGF expression in tissues is recommended. Type of breast cancer is another factor which has been proposed in this regard. It is generally assumed that the VEGF is more severely expressed in IDC cases comparing with other type (Turashvili *et al.*, 2005; Hieken *et al.*, 2001; Lee *et al.*, 1998).

We also confined the study to the IDC cases. Other confounding factors such as the menopause status and presence or absence of estrogen receptor are also discussed here (Gunningham *et al.*, 2001; Soufla *et al.*, 2006).

Differences in technical experiences and sensitivity of methods employed may also justify the present heterogeneity (Hoar *et al.*, 2003).

CONCLUSION

In patients with IDC of breast, it seems that the status of VEGF expression is not associated with

involvement of axillary lymph nodes and hence, may not be of prognostic value. Further more controlled evaluations with larger sample sizes are recommended.

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