ORIGINAL ARTICLE

Vascular endothelial growth factor expression and significance in different grades of the Breast Imaging Reporting and Data System*

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Abstract

Objective   Mammography is the only modality proven to reduce mortality in breast cancer, and ultrasonography is a well-known adjunct to mammography screening. The Breast Imaging Reporting and Data System (BI-RADS) classification is a practical tool and is correlated with histopathology and combined use with triple assessment (examination, imaging, and biopsy) of palpable diagnostic cases. This study aimed to investigate the relationship between vascular endothelial growth factor (VEGF) expression and different grades of BI-RADS in breast cancer.

Methods   Ninety-six patients with breast carcinoma were evaluated using BI-RADS by ultrasonography, mammography, and a combination of both modalities. In the combined imaging assessment, BI-RADS 1–4a grade was considered when the score of ultrasonography and mammography was lower than 4a, and BI-RADS 4b–5a grade was considered when the score of ultrasonography and mammography was higher than 4a. Immunohistochemical Ultra Sensitive™ S-P method was employed to evaluate the expression of VEGF in 96 patients. Fifty patients with benign breast disease were selected as the control group. The relationship between VEGF expression and different grades of BI-RADS and that between VEGF expression and other standard prognostic parameters associated with invasive breast cancer, such as size, grade, cancer stage, and metastasis were analyzed.

Results   The sensitivities of ultrasonography and mammography alone was 74.0% and 84.4%, respectively; However, the sensitivity of their combination increased to 90.6%. The positive rates of VEGF in invasive breast cancer BI-RADS 4b–5 (59/87, 67.8%) were higher than those in BI-RADS 4a (3/9, 33.3%, $P < 0.05$) and benign breast disease tissues (BI-RADS 1–4a, 11/50, 22.0%) ($P < 0.05$). There was a positive correlation between VEGF overexpression and BI-RADS 4b–5, histological grade (III), lymph node metastasis, and distant metastasis of invasive breast cancer. VEGF expression was not related to the age and size of the tumor in each group ($P > 0.05$).

Conclusion   There was a positive correlation between VEGF overexpression and BI-RADS 4b–5 grade. The overexpression of VEGF might be an important biological marker for the invasion and metastasis of breast cancer.

Key words: breast cancer; vascular endothelial growth factor (VEGF); BI-RADS; ultrasonography; mammography

Breast cancer is the most common form of cancer in women and the second leading cause of death due to malignant disease in women, following lung cancer [1–2]. Recently, there has been an upward trend in the incidence of breast cancer in our country, and it tends to develop at a younger age [3–4]. Furthermore, breast cancer has become one of the most common malignant tumors in women and is therefore one of the most significant threats to a woman's health [3–4]. The American College of Radiology (ACR) Breast Imaging Reporting and Data System (BI-RADS) classification is a practical tool and has correlation with histopathology and combined uses with triple assessment (examination, imaging and biopsy) of palpable diagnostic cases. Mammography is widely used in the diagnosis and screening of breast cancer and the only modality that has been proven to reduce mortality in breast cancer, and with recent technological advancements, ultrasonography in breast examination plays an important role in breast cancer detection, diagnosis, needle biopsy, and operation method selection [5]. The development of ultrasonography has made the early detection of small lesions in the breast possible, which has been shown to reduce mortality in breast cancer [3, 5]. When the tumor diameter is > 2 mm, new vessels will be generated to provide essential oxygen and nutrition to the tumor [6–7]. New vessels that promote tumor growth and metastasis are controlled by several positive and negative factors [6]. The vascular endothelial growth factor (VEGF), which promotes angiogenesis and proliferation of endothelial cells, exerts an important effect in the genesis, development, metastasis, and recurrence of various tumors. To date, there are few studies on the relationship between VEGF expression and different grades of BI-RADS. This study aimed to evaluate the relationship between VEGF expression and different grades of BI-RADS in breast cancer.

Materials and methods

Patients

Ninety-six patients diagnosed with invasive breast carcinoma were evaluated using the BI-RADS by ultrasonography, mammography, and a combination of both modalities. Complete clinical and follow-up data were confirmed by surgery and pathology (Table 1). We excluded cases in which both ultrasonography and mammography were not performed. Preoperatively, the BI-RADS breast lesions detected by routine ultrasonography and mammography were analyzed. Postoperatively, the breast lesions were diagnosed as benign and malignant lesions according to the pathological results. The ages of the 96 patients diagnosed with breast carcinomas ranged from 28 to 74 years (mean, 46.8 years). All patients underwent excision surgery at Rizhao

Table 1 Clinicopathological factors of breast cancer (n = 96)

Groups	n
Age at diagnosis (years)	
≤ 50	39
> 50	57
Tumor size (cm)	
≤ 2	31
> 2	65
Histoligical grade	
I + II	67
III	29
Lymph node metastasis	
Present	61
Absent	35
Distant metastasis	
Present	25
Absent	71

People's Hospital from January 2016 to December 2018 and were evaluated using BI-RADS by ultrasonography, mammography, and a combination of both modalities. Fifty patients with benign breast disease were included in the control group, with age ranging from 28 to 71 years (mean, 48.8 years).

Methods

All 96 patients with invasive breast carcinomas were evaluated using BI-RADS by ultrasonography, mammography, and a combination of both modalities. Patients were divided into two groups based on ultrasonography and mammography findings: ACR BI-RADS 1–4a and 4b–5 groups. If the patient underwent more than one imaging examination before tissue biopsy, the latest imaging result was analyzed. In patients with bilateral biopsies or more than one biopsy in one breast, the most serious result was considered. In the combined imaging assessment, BI-RADS 1–4a grade was considered when the score of ultrasonography and mammography was lower than 4a, and 4b–5 grade was considered the score of ultrasonography and mammography was higher than 4a. The relationship between VEGF expression and different grades of BI-RADS in breast cancer and that between VEGF expression and other standard prognostic parameters associated with invasive breast cancer, such as size, grade, cancer stage, and metastases, were analyzed.

Imaging protocols

Ultrasonograms and mammograms were interpreted by experienced technologists, and the findings were reported by two experienced radiologists based on ACR BI-RADS grades. All ultrasonography examinations included real-time bilateral whole-breast and power Doppler blood flow scans using ultrasound machines (IU Elite Medical System, Philips, USA), with linear probes

measuring 5–12 MHz. Diagnostic mammograms were obtained in standard craniocaudal and mediolateral oblique views by well-trained technologists using digital mammography machines with full-field digital mammograms (Senographe 2000D, GE, USA). Mammography and breast ultrasound findings were classified using the BI-RADS into five grades: BI-RADS 1, breasts where no pathological lesions are seen; BI-RADS 2, benign findings; BI-RADS 3, probably benign findings; BI-RADS 4, lesions suspicious for malignancy; BI-RADS 4, divided into three sublevels of 4a, 4b, and 4c; BI-RADS 5, lesions highly suspicious for malignancy or malignant lesion. A lesion graded lower than BI-RADS 4a is considered a benign lesion, while a lesion graded higher than 4a is considered a malignant lesion.

Pathology

Breast tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin at 4 °C for 24 h. Tissue sections of 5 μm thickness were deparaffinized and rehydrated using standard procedures. The specimens were examined under a binocular-dissecting microscope. The pathological diagnosis was independently verified by two pathologists using histological methods, and pathological grading was determined according to the current 2012 World Health Organization classification system (WHO 2012)[6]. Immunoreactions were processed to detect VEGF expression in 96 patients using the Ultra SensitiveTM S-P Kit (Maixin-Bio, China) according to the manufacturer's instructions, and signals were visualized using the DAB substrate, which stains the target protein yellow. Negative controls were used. The primary antibody was replaced with PBS containing 0.1% bovine serum albumin at the same concentration as the primary antibody. The positive controls were tissues known to express the antigen being studied. VEGF was localized in the cytoplasm and membrane. Cells were classified according to the positive rate and color intensity as follows: negative, number of positive cells < 25%; positive, brown particles, number of positive cells ≥ 25%. The pathological reading was determined for each biopsy slide with an overall pathological diagnosis determined for each subject. The tumor grade was determined according to the modified Bloom-Richardson score. Fifty patients with benign breast disease were included in the control group.

Statistical analysis

SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Enumeration data were analyzed using the chi squared (χ^2) test. A P value < 0.05 was considered statistically significant.

Table 2 Results of BI-RADS assessments of different breast cancer detection methods

BI-RADS assessment	No. of patient		Total
	1–4a	4b–5	
Mammography alone	15	81	96
Ultrasonography alone	25	71	96
Combination*	9	87	96

*Combined mammography and ultrasonography: BI-RADS 1–4a grade was considered when the score of mammography and ultrasonography was lower than 4a, and 4b–5a category was considered when the score of mammography and ultrasonography was higher than 4a

Results

BI-RADS finding

The sensitivity of ultrasonography alone was 74.0%, and that of mammography alone was 84.4%. However, the sensitivity of the combination of ultrasonography and mammography increased to 90.6% (Table 2). Factors such as tumor edge, contrast mode, lobular sign, prick sign, vascular distortion, skin thickening in areola area, and score of elastography were related to the benign and malignant features of breast lesions (Table 3). High BI-RADS grade on ultrasonography was often indicated by breast hypoechoic mass, heterogeneous internal echo, punctate calcification with strong echo and unclear boundary irregular margin, and lobular sign. Elastography showed that the tubercle was hard. Color Doppler flow imaging (CDFI) showed blood flow signal in the border area of the nodule or short rod blood flow signal in the mass (Fig. 1). High BI-RADS grade on mammography was indicated by breast mass, irregular margin, lobular sign, prick sign, vascular distortion, nipple depression, and skin thickening in areola area (Fig. 2).

VEGF expression in different BI-RADS grade groups

The positive rates of VEGF in invasive breast cancer BI-RADS 4b–5 (59/87, 67.8 %) were higher than those in breast cancer BI-RADS 4a (3/9, 33.3%) and benign breast disease tissues (BI-RADS 1–3, 11/50, 22.0%) (P < 0.05) (Table 4). There was a positive correlation between VEGF overexpression and BI-RADS 4b–5 (P < 0.05).

Relationship between VEGF expression and clinicopathological parameters

The positive rates of VEGF in invasive breast cancer were 64.6% (62/96), which were higher than those in benign lesion tissues (22.0%, 11/50) (P < 0.05) (Table 5). There were significant differences in the VEGF expression between histological invasive breast cancer groups with lymph node metastasis, distant metastasis, and recurrence and those without (P < 0.05). There was a positive correlation between VEGF overexpression and

Table 3 BI-RADS findings in patients with breast cancer (*n* = 96)

Groups	Total
Ultrasonography	
Mass Echo homogeneous	
Yes	23
No	73
Boundary clear	
Yes	52
No	44
Ring strong echo around the mass	
Yes	21
No	75
Edge regular	
Yes	29
No	67
Peripheral tissue echo slight	
Yes	31
No	65
CDFI short rod blood flow signal in the mass	
Yes	49
No	47
Elastic imaging showed that the tubercle was hard	
Yes	18
No	78
Mammography	
Breast mass	
Yes	77
No	19
Edge regular	
Yes	17
No	79
Margin clear	
Yes	23
No	73
Lobular sign	
Yes	47
No	49
Prick sign	
Yes	28
No	68
Vascular distortion	
Yes	34
No	62
Nipple depression	
Yes	31
No	65
Skin thickening in areola area	
Yes	28
No	68

histological grade III, lymph node metastasis, and distant metastasis in invasive breast cancer. However, there was no significant difference in VEGF expression with respect to age (≤ 50 years *vs* > 50 years) and tumor diameter (≤ 2 cm *vs* > 2 cm) (*P* > 0.05).

Discussion

Breast cancer is one of the most common malignant tumors, and its morbidity rate is increasing annually [8]. The overall BC incidence has been increasing in China, with an earlier age of onset compared with Western countries and a peak incidence rate at age 50 years [9]. Early detection, diagnosis, and treatment play an important role in the prognosis of breast cancer [10]. As a result, numerous studies worldwide have sought to determine the most effective strategies to conduct early diagnosis, treat breast cancer, assess therapeutic effects, correctly evaluate prognosis, and identify postoperative recurrence in patients. Imaging examination is important in the detection and diagnosis of breast cancer [11]. The ACR BI-RADS classification is a practical tool and has correlation with histopathology and combined uses with triple assessment (examination, imaging, and biopsy) of palpable diagnostic cases. The BI-RADS was established by the ACR to standardize mammographic reporting in 2003 [12]. Five levels are included in the BI-RADS, and level 4 is divided into three sublevels: 4a, 4b, and 4c. A lesion categorized as lower than 4a is considered a benign lesion, while a lesion categorized higher than 4a is considered a malignant lesion. The ACR BI-RADS provides standardized descriptors of imaging features of breast lesions, is helpful in predicting benign or malignant potential, and can be used globally. Mammography is an accurate, relatively inexpensive, and convenient technique; therefore, it is an important method used in the diagnosis of breast cancer. Mammography is the only modality that has been proven to reduce mortality in breast cancer [3], and ultrasonography is a well-known adjunct to mammography screening [5]. Routine mammography and sonographic manifestations of BI-RAD 4 breast lesions tend to have a certain degree of overlapping and are sometimes difficult to assess [13]. Thus, it is difficult to identify the nature of such lesions in the clinic. In our study, the sensitivity of detection using mammography was 84.4%, and that of ultrasonography was 74.0%; however, the sensitivity detection using the combination of ultrasonography and mammography increased to 90.6%. Our results reveal that the characteristics of irregular margin, lobular sign, prick sign, vascular distortion, nipple depression, and skin thickening in the areola area are important early signs of breast cancer and may sometimes be the sole sign of malignancy. Our study showed that the tufted apical calcification detection rate can be further improved by mammography than ultrasonography, and mammography is regarded the gold standard for the detection and characterization of microcalcifications. However, the marked improvement of current high-frequency transducer technology has yielded high spatial resolution, allowing better and
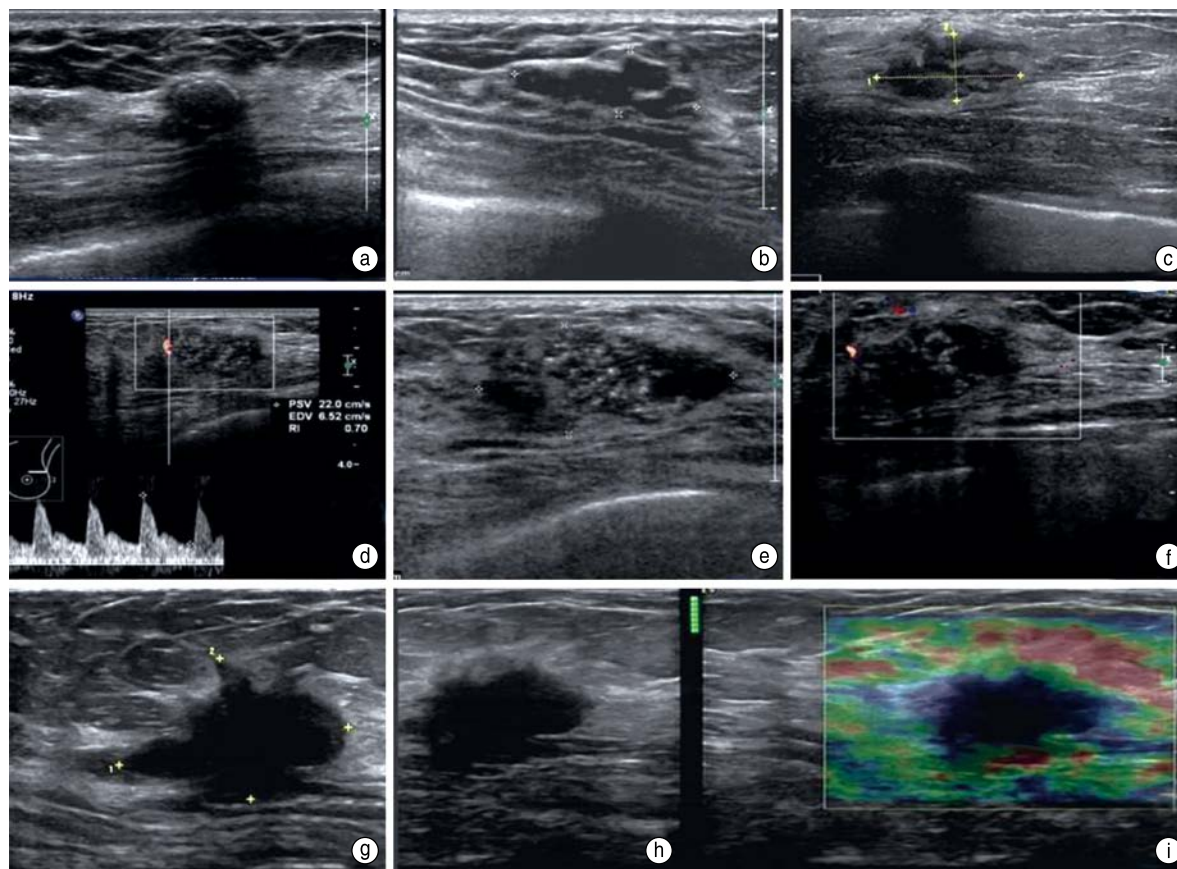
Fig. 1 BI-RADS on ultrasonography. (a) BI-RADS 3 (benign lesion). The echo inside the mass was not homogeneous, boundary was still clear, and ring echo around the mass was strong; (b) BI-RADS 4a (benign lesion). The internal echo of the mass was not homogeneous and the boundary was still clear, but the edge was irregular; (c) BI-RADS 4b (invasive ductal carcinoma II). The echo inside the mass was not homogeneous, boundary was not clear, edge was irregular, and echo behind the mass was weak; (d, e, and f) BI-RADS 4c (invasive ductal carcinoma III with ductal carcinoma in situ). Hypoechoic mass, heterogeneous internal echo, punctate calcification with strong echo, and unclear boundary were observed. CDFI showed short rod blood flow signal in the mass; (h and i) BI-RADS 5 (invasive ductal carcinoma II). The shape of the nodule was irregular, edge was not smooth, burr sign was visible, internal echo was weak, punctate strong echo was visible, signal behind the nodule was attenuated, and surrounding gland structure was twisted. Elastography showed that the tubercle was hard. CDFI showed the blood flow signal in the border area of the nodule

more frequent visualization of breast microcalcifications [3]. Calcification within the breast and simple clustered calcification are important early signs of breast cancer and may sometimes be the sole sign of malignancy [3]. In this study, high BI-RADS grade on ultrasonography was often indicated by breast hypoechoic mass, heterogeneous internal echo, punctate calcification with strong echo and unclear boundary irregular margin, and lobular sign. Elastography showed that the tubercle was hard. CDFI showed blood flow signal in the border area of the nodule or short rod blood flow signal in the mass.

However, even after curative resection, tumor recurrences are likely to assume different forms in various organs. The prediction of risks for recurrences and recurrence patterns after surgery could help the development of better follow-up programs and appropriate treatment strategies for patients with breast cancer. Additionally, the prognosis after resection has remained unsatisfactory due to a high incidence of cancer lymph node metastases and cancer recurrence. The identification of variables in breast tumor biology may lead to a more precise assessment of outcome and response to therapy. The development of prognostic markers that can accurately predict outcome is crucial in identifying patients who could benefit from aggressive therapy. VEGF, one of the key factors to promote tumor angiogenesis and with the strongest function and highest specificity, can not only promote the proliferation of endothelial cells but also regulate and participate in angiogenesis. Due to an intimate association with genesis, development, metastasis, and infiltration of breast cancer, it is an important indicator in evaluating metastasis and infiltration of breast cancer in the clinic.

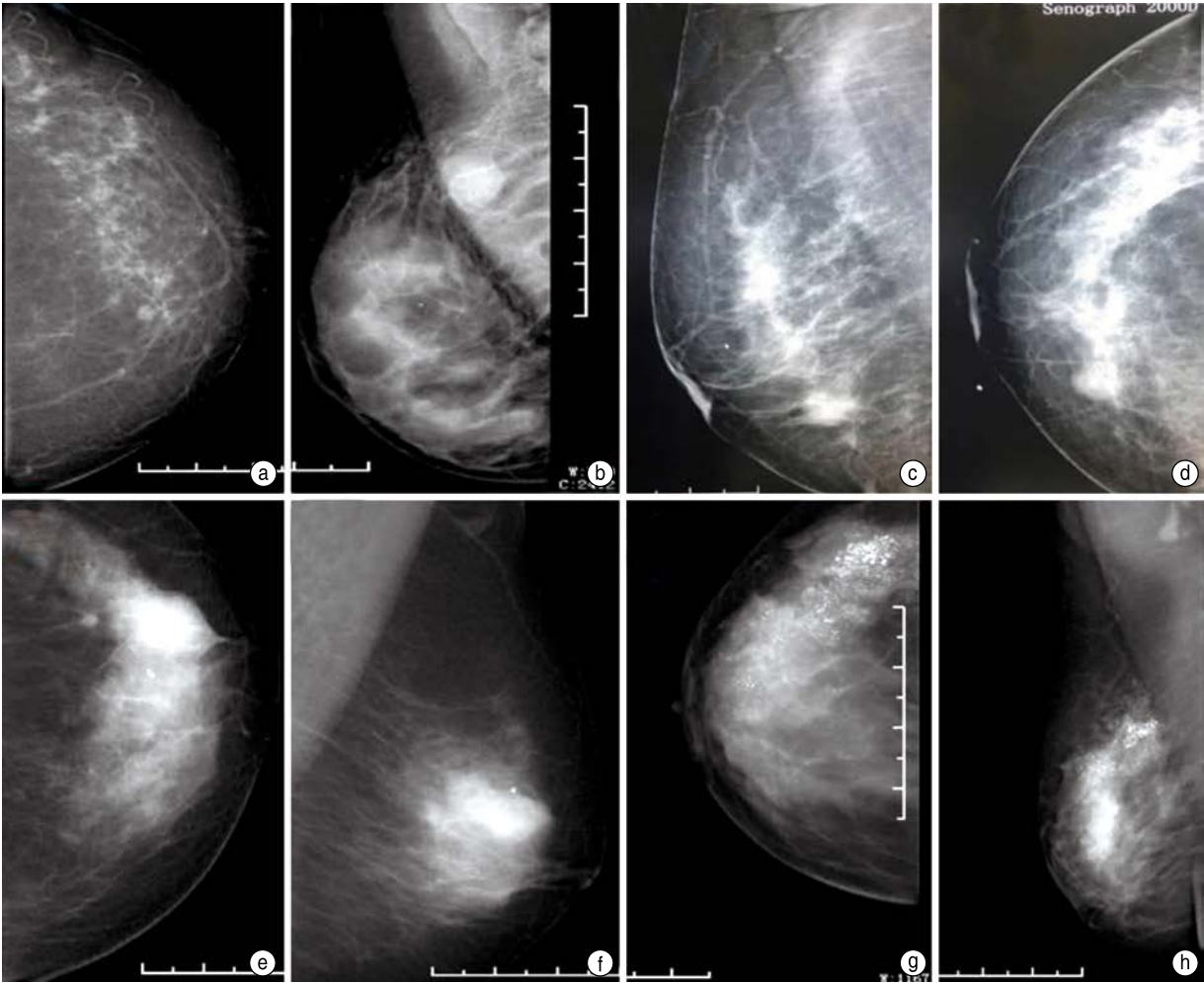In our study, the positive rates of VEGF in breast

Fig. 2 BI-RADS on mammography. (a) BI-RADS 3 (benign lesion), CC, the boundary of the mass was clear; (b) BI-RADS 4a (benign lesion), MLO, there was a nodule near the pectoralis major muscle in the upper right breast with clear margin; (c and d) BI-RADS 4b (invasive ductal carcinoma II), (c) CC and (d) MLO. Inferior intramammary nodule, unclear margin, and lobular sign; (e and f) BI-RADS 4c (invasive ductal carcinoma II), (e) CC and (f) MLO. Left lateral superior breast nodule, uneven density, unclear margin, and visible lobular sign; (g and h) BI-RADS 5 (invasive ductal carcinoma III), (g) CC and (h) MLO. The density of the right breast was obviously increased, the margin was unclear, and there was scattered cluster needle tip calcification

Table 4 VEGF expression of in different BI-RADS groups (*n* = 96)

Pathologic diagnosis	BI-RADS groups	VEGF express −	VEGF express +	Total
Invasive carcinoma	4b–5	28	59	87
	4a	8	3	9
Benign leisions	1–3	39	11	50

Table 5 VEGF expression in different groups

Groups	VEGF express −	VEGF express +	Total
Breast carcinoma	34	62	96
Benign breast leisions	39	11	50

cancer were higher than those in benign lesion tissues, and there were significant differences in the VEGF expression between histological breast cancer groups with lymph node metastasis, distant metastasis, and recurrence and those without. These research results revealed that the VEGF expressions in the patients with lymph node metastasis were markedly higher than those without lymph node metastasis, and the difference was statistically significant. The results suggested that, with

increasing pathological stage, the VEGF levels in the observation group gradually increased, and the statistical significance was remarkable. In the study, significantly higher VEGF expression was found in tumors with lymph node metastasis, advanced stage, and recurrence. When tumors were divided into grade I–II and grade III, high VEGF expressions were also significantly associated with advanced grade (III). However, there was no difference in the VEGF expressions with respect to age at diagnosis

(≤ 50 years *vs* > 50 years) and tumor size (≤ 2 cm *vs* > 2 cm). Additionally, our studies suggested that the positive rates of VEGF in invasive breast cancer BI-RADS 4b–5 were higher than those in breast cancer BI-RADS 4a and benign breast disease tissues (BI-RADS 1–3). There was a positive correlation between VEGF overexpression and BI-RADS 4b–5. VEGF that is capable of promoting angiogenesis exerts an important effect in the processes of genesis, development, metastasis, and recurrence of various tumors. In the process of tumor genesis and development, tumor regenerative capillaries capable of providing nutrients to tumor cells and favorable conditions for distal metastasis are the precondition to induce local growth, infiltration, and distal metastasis of malignant tumors; hence, how to inhibit tumor angiogenesis is a new research hotspot at present [14]. Due to the intimate association between genesis, development, metastasis, and infiltration of breast cancer, VEGF is an important indicator of metastasis and infiltration of breast cancer in the clinical setting. However, further study is needed to understand the exact pathogenic mechanism.

Conclusions

Our results reveal that there is a positive correlation between VEGF overexpression and BI-RADS 4b–5 in breast cancer. Increased VEGF expressions are associated with tumor progression, invasion, and metastasis. The overexpression of VEGF might be an important biological marker for invasion, metastasis, and poor differentiation, especially in higher-grade lesions and metastatic disease.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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