Original Article

موضوع أصيل

PROGNOSTIC ROLE OF CIRCULATING IL-17 IN BREAST CANCER AND ITS ASSOCIATION WITH VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) SERUM LEVELS

الدور الإنذاري للمستويات الدورانية للانترلوكين 17 (IL-17) في سرطان الثدي وعلاقته مع التراكيز المصلية لعامل النمو البطاني الوعائي (VEGF)

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ملخص البحث

الخلفية: يعدّ الانترلوكين 17 (IL-17) Interleukin-17 سيتوكيناً التهابياً تنتجه خلايا CD4+T. أعطت الدراسات التي تناولت الدور الإنذاري لـ IL-17 في سرطان الثدي نتائج متناقضة. في هذه الدراسة تمّ التحرّي عن المستويات المصلية لـ IL-17 لدى مريضات سرطان الثدي مع تقييم مدى ارتباط هذه المستويات مع المعايير المرضية والسريرية. إضافةً إلى ذلك تمت دراسة العلاقة بين التراكيز المصلية لكل من IL-17 وعامل النمو البطاني الوعائي vascular endothelial growth factor (VEGF).

المواد والطرائق: تم سحب 3 مل من الدم المحيطي من 41 مريضة سرطان ثدي و28 امرأة صحيحة، ثمّ جرى تقييم تراكيز IL-17 وVEGF في المصل لتحديد مدى ارتباطها مع تطور الورم. تم تحديد المستويات المصلية باستخدام طريقة مقايسة الممتز المناعي المرتبط بالإنزيم enzyme-linked immunosorbent assay (ELISA).

النتائج: بينت الدراسة أن تراكيز IL-17 في المصل لدى مريضات سرطان الثدي أعلى بشكل هام إحصائياً مقارنة مع مستوياته لدى الصحيحات، كما ارتبطت تراكيز IL-17 لدى المريضات مع تشكّل أوعية جديدة للورم ومع النقائل إلى العقد اللمفية، مما يشير إلى إمكانية وجود علاقة بين IL-17 وتطور الورم. كما تبيّن وجود علاقة إيجابية قوية بين التراكيز المصلية لكل من IL-17 وVEGF لدى مريضات سرطان الثدي.

الاستنتاجات: تشير الدراسة الحالية إلى ارتباط المستويات الدورانية لـ IL-17 بشكل قوي مع تطور سرطان الثدي، مما يمكن أن يتيح استخدام تراكيز IL-17 لتمييز مريضات سرطان الثدي المترافق مع إنذار سيئ واللواتي قد يستفدن من تدابير علاجية أكثر شدّة.

ABSTRACT

Objective: *Interleukin-17 (IL-17) is a CD4 T-cell-derived proinflammatory cytokine. Studies investigating the prognostic effect of circulating IL-17 in breast cancer have given inconsistent findings. In this study, we investigated the serum level of IL-17 in breast cancer patients, and evaluated whether circulating IL-17 is associated with clinicopathological parameters. Furthermore, we studied the relationship between IL-17 and vascular endothelial growth factor (VEGF) serum levels.*

Methods: *A 3 ml of peripheral blood were obtained from 41 patients with breast cancer and 28 healthy women. We assessed the oncentration of IL-17 and VEGF in blood serum to determine whether it correlates with the disease progression. Serum levels were quantified by enzyme-linked immunosorbent assay (ELISA) method.*

Results: *Concentration of IL-17 was found to be significantly increased in breast cancer patients*

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compared with control group. The circulating level of IL-17 in breast cancer patients was associated with the angiogenesis and lymph node metastasis, suggesting that IL-17 was related to tumor progression. Moreover, the serum level of IL-17 strongly and positively correlated with that of VEGF in cancer patients.

Conclusions: *The present study indicates that IL-17 serum concentration are strongly associated with breast cancer progression. It was feasible that it can be used to identify patients with a poor prognosis who may benefit from more aggressive management.*

INTRODUCTION

Breast cancer is responsible for the majority of women deaths due to cancer worldwide. Therefore, it is important to clarify the mechanisms involved in the development of breast cancer. Angiogenesis has received a lot of attention due to its influence on the tumour grade, metastasis, and therefore patients' prognosis; over-expression of its hallmark cytokine, vascular endothelial growth factor (VEGF), is well known amongst various malignant cancers often with poorer outcomes.[1-5] Tumors or interacting stromal cells may cause imbalance to increase elaboration of angiogenic inducers or decrease production or effects of angiogenic suppressors. Identification of endogenous angiogenesis stimulators or inhibitors is an area of great interest.[6]

It has been established that cancer can be promoted or exacerbated by inflammation and infection. Chronic inflammation is a major driving force in tumor development.[7,8] IL-17 (interleukin-17) is a proinflammatory cytokine generated by Th17 cells, and has a dual and contradictory role in the cancer process. IL-17 can activate a cell-mediated cytotoxicity against tumor cells, that can in turn inhibit tumor progression, while some researchers have shown that IL-17 has a critical role in facilitating the angiogenesis.[9-11] Recently, it has been reported that IL-17 promotes tumor growth through angiogenesis in mice.[12]

Therefore, the characteristic biologic activities of IL-17 prompted us to further investigate the association of serum IL-17 with breast cancer in an effort to define the role of circulating IL-17 as a prognostic factor in breast cancer. Furthermore, we studied the relationship between pretreatment serum IL-17 and VEGF levels, and whether serum IL-17 levels can predict angiogenesis of breast cancer.

METHODS

The present study was conducted during a period from December 2018 to September 2019 at Al-Muwasah Teaching Hospital (Damascus). Informed written consent was obtained from each patient prior to the specimen collection, 41 patients with breast cancer were enrolled in the present study. The age range and the mean age of patients were 21-75 and 49.6±11.7 years, respectively. Control group comprised 28 healthy women with age range 26-79 years and mean age 39.4±12.7 years. Controls were selected among individuals who had no clinical evidence and history of malignancy or autoimmune disorders. Pathological data [including: tumor grade, lymph node status, angiogenesis] were obtained from medical records of

Factor		Frequency	Percentage
Tumor grade	I	7	17.1
	II	22	53.6
	III	12	29.3
Lymph node metastasis	Metastasic	18	43.9
	Non-metastasic	23	56.1
Angiogenesis	Angiogenic	14	34.1
	Non-angiogenesis	27	65.9

Table 1. Histopathologic information in breast cancer patients.

patients, validated by an experienced histopathologist, and summarized in Table 1.

Three mL of blood samples were obtained from patients (on the same day of surgery) and healthy women according to the internal review and the Ethics Boards of the Syrian public hospitals. Blood serum was stored in a freezer at -20°C.

Interleukin-17 and VEGF concentrations were determined in serum samples by enzyme-linked immunosorbent assays (ELISAs). ELISA reagents are commercially available in assay kits (Bender MedSystems, Austria).

Statistical differences were analyzed by Student's *t* test and ANOVA test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Concentrations of IL-17 and VEGF were determined in serum from patients with breast cancer and compared with healthy controls. Although comparison of 2 given groups indicated an increase of IL-17 in cancer patients, the increase was of no statistical significance ($p>0.05$; Table 2).

VEGF levels was found to be significantly higher in cancer patients compared with the control group ($p=0.001$; Table 2). In addition, a positive significant correlation between serum concentrations of IL-17 and VEGF was demonstrated ($p=0.001$, $r=0.813$; Figure 1).

To evaluate the biological significance of IL-17 in patients with breast cancer, we investigated the association of serum levels of IL-17 with clinicopathological factors (Table 3). IL-17 levels in patients with breast cancer were higher in positive groups of angiogenesis ($p=0.001$) and lymph node metastases ($p=0.038$) than in negative groups. On the other hand, no significant association was recognized between levels of IL-17 and tumor grade ($p>0.05$).

The distribution of the VEGF serum concentration according to clinicopathological parameters of breast cancer is shown in Table 4. A statistically significant increase was seen only in serum levels of VEGF in patients with angiogenic cancer and those with metastasis to lymph nodes ($p<0.05$, Table 4), whereas VEGF concentrations were not significantly correlated with the grade of the disease ($p>0.05$, Table 4).

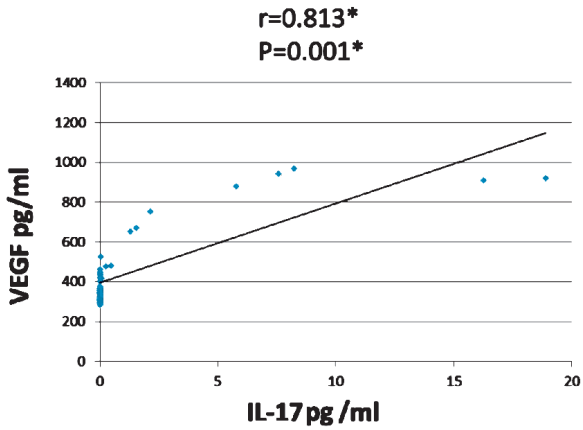
Patients		Number	Mean (pg/ml)	SD (pg/ml)	p-value
IL-17	Cancer	41	1.53	4.16	0.057
	Control	28	0	0	
VEGF	Cancer	41	457.32	204.01	0.001
	Control	28	265.21	37.47	

Table 2. comparison of IL-17 and VEGF concentration in serum samples of patients.

Number		Mean (pg/ml)	SD (pg/ml)	p-value
Angiogenic	14	4.46	6.26	0.001[a]
Non-angiogenesis	27	0	0.02	
Metastasic	18	3.04	5.87	0.038[a]
Non-metastasic	23	0.35	1.23	
Grade I	7	0.32	0.64	0.886[b]
Grade II	22	1.94	4.55	
Grade III	12	1.36	4.69	

[a]P-value of student *t* test as appropriate. [b]P-value of ANOVA test as appropriate.

Table 3. The difference in median levels of serum IL-17 (pg/ml) in breast cancer patients according to clinicopathological parameters.



*There was a positive association between the levels of IL-17 and VEGF in cancer patients. Levels were detected using ELISA and evaluated as pg/ml. IL: Interleukin, VEGF: vascular endothelial growth factor.

Figure 1. Correlation between serum levels of IL-17 and VEGF in patients with breast cancer.

Number		Mean (pg/ml)	SD (pg/ml)	p-value
Angiogenic	14	360.85	59.63	0.001[a]
Non-angiogenesis	27	643.36	253.68	
Metastasic	18	403.91	135.28	0.002[a]
Non-metastasic	23	643.3	281.36	
Grade I	7	403.75	168.68	0.814[b]
Grade II	22	470.59	235.96	
Grade III	12	440.83	160.89	

[a]P-value of student *t* test as appropriate. [b]P-value of ANOVA test as appropriate.

Table 4. The difference in median levels of serum VEGF (pg/ml) in breast cancer patients according to clinicopathological parameters.

DISCUSSION

In this study, we quantitatively analyzed the circulating levels of both IL-17 and VEGF in breast cancer patients. As an essential process in breast cancer development and progression, angiogenesis provides not only oxygen and nutrients for tumor growth but also more opportunities for tumor cells to migrate and metastasize.[13] Moreover, many studies have revealed that angiogenesis is an important indicator of poor prognosis in breast cancer.[14] There are many endogenous factors that facilitate angiogenesis, and the VEGF/VEGFR family includes the strongest growth factor that directly acts upon endothelial cells in angiogenesis.[15]

In the present work, there was a significant increase in serum VEGF concentration of patients with breast cancer compared with healthy women. Furthermore, a high serum VEGF concentration was significantly associated with the presence of lymph nodes metastasis. These findings are similar to others, both in breast cancer16,17 and other tumor types.[18]

IL-17 was originally identified as a proinflammatory cytokine, and previous studies have also shown that inflammation is linked to cancer progression.[19] Therefore, it is reasonable to speculate that IL-17 may correlate with development of cancer. Indeed, we showed in the present study that the level of IL-17 was significantly higher in serum of cancer patients with angiogenesis and those with lymph node metastasis. This result was consistent with previous data that IL-17 promotes angiogenesis through stimulation of vascular endothelial cell migration and cord formation, resulting

in tumor progression.[12] In addition, Benevides et al. in 2015 uncovered that the mechanism by which the proinflammatory cytokine IL-17 promotes metastatic mammary primary tumor progression is neutrophil dependent.[20]

Our data also demonstrated a positive correlation between the IL-17 and VEGF serum concentrations. This finding is consistent with previous reports that IL-17 may shift the local balance between angiogenic and angiostatic factors toward conditions that promote angiogenesis and tumor development.[21] IL-17 up-regulates production of a variety of proangiogenic factors, such as vascular endothelial growth factor (VEGF), prostaglandin E1 (PGE1) and PGE2, and macrophage inflammatory protein-2 (MIP-2), by fibroblasts as well as tumor cells.[12]

CONCLUSIONS

Our findings showed that IL-17 serum concentrations were associated with breast cancer metastasis and angiogenesis. Moreover, we observed a positive correlation between the IL-17 and VEGF serum levels. Therefore, IL-17 may be developed as a potential prognostic biomarker for breast cancer.

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