

Association of serum interleukin-10, interleukin-17A and transforming growth factor- α levels with human benign and malignant breast diseases

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Abstract. Interleukin-10 (IL-10), interleukin-17A (IL-17A) and transforming growth factor α (TGF- α) have been implicated in the progression of breast cancer. However, the diagnostic and prognostic roles of these cytokines in ductal carcinoma remain unclear. The present study therefore aimed to determine the serum levels of IL-10, IL-17 and TGF- α in subjects with benign and malignant breast diseases and to evaluate the clinical significance of these cytokines in ductal carcinoma. Pre-operative serum samples were collected from 378 patients with breast disease and 70 healthy subjects. IL-10, IL-17A and TGF- α levels were measured using ELISA. Serum levels of these cytokine in patients with different breast diseases were evaluated. Furthermore, correlations between levels of these cytokines and the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) in ductal carcinoma were determined. The results demonstrated that serum levels of IL-10 and IL-17A were

significantly increased in subjects with atypical hyperplasia and ductal carcinoma. Furthermore, IL-10 and IL-17A levels were increased in patients with a more serious clinical tumor stage and tumors that were ER⁻ and PR⁻. Furthermore, high serum levels of TGF- α were associated with HER2⁺ tumors. A strong positive correlation was identified between TGF- α and IL-17A levels. Therefore, the results of the current study revealed that elevated serum IL-10, IL-17A and TGF- α levels are strongly associated with ductal carcinoma, specifically with tumor stage. High serum levels of IL-10 and IL-17A were also associated with the negative expression of ER and PR in ductal carcinoma, and high serum levels of TGF- α were associated with the positive expression of HER2 in ductal carcinoma. Thus, serum cytokine levels may be measured to identify patients with a poor prognosis who may benefit from more aggressive management and treatment.

Introduction

Human benign and malignant breast diseases, including atypical hyperplasia, papilloma, fibroma and ductal carcinoma, are usually associated with inflammation (1,2). Benign breast conditions are common and the majority of breast changes are not cancer; however, certain benign diseases increase the risk of breast cancer, including atypical hyperplasia, which is a potential precursor of cancer (3). Cells involved in the inflammatory response are attracted by cytokines and chemokines and may promote the onset and progression of breast diseases (4-7). Inflammation mediates the initiation and growth of breast cancer and cytokines serve a very important role during this process (5,8).

Previous studies have indicated that pro-inflammatory cytokines, including interleukin (IL) 17A and transforming growth factor α (TGF- α) are involved in the initiation and progression of mammary diseases (5,9,10). IL-17A is produced by activated T cells (9). The number of IL-17A-producing tumor-infiltrating lymphocytes (TILs) is increased in patients with breast cancer,

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Abbreviations: IL, interleukin; TGF- α , transforming growth factor α ; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TILs, tumor-infiltrating lymphocytes

Key words: interleukin-10, interleukin-17A, transforming growth factor- α , human benign and malignant breast diseases

particularly in estrogen receptor-negative (ER⁻) tumors, these cells are indicative of a poor prognosis for the patient (9,11,12). TGF- α is secreted by various types of cells and stimulates their proliferation, differentiation and development (10). Previous studies have demonstrated that TGF- α levels are correlated with different types of tumor, including mammary, squamous and renal tumors (10,13). Anti-inflammatory cytokines are also very important in tumor development; for example IL-10, which is a potent anti-inflammatory cytokine, promotes the formation of a microenvironment, which inhibits anti-tumor immune response and promotes the growth of cancer cells (14-16). It has been demonstrated that serum levels of IL-10 are higher in patients with breast cancer than in healthy subjects and IL-10 is also overexpressed in ER⁻ breast cancer, compared with ER⁺ breast cancer (17). Several studies have identified the presence of these cytokines in breast, liver and gastric cancer (13,17-19); however, it remains unclear whether serum levels of these cytokines are correlated with clinical tumor stage. Additionally, it is unclear whether the expression of human epidermal growth factor receptor 2 (HER2) in tumors is correlated with the serum levels of these cytokines. HER2 is a tumor antigen that is overexpressed in breast cancer and is a very important biomarker for predicting the prognosis of patients with breast cancer.

To fully understand the significance of IL-10, IL-17A and TGF- α during the progression of breast cancer, the serum levels of these cytokines in patients with mammary diseases, including atypical hyperplasia, papilloma, fibroma and ductal carcinoma, were compared to those in healthy women. It was also determined whether there was a correlation between levels of these cytokines and clinical tumor stage, as well as with ER, progesterone receptor (PR) and HER2 antigen expression.

Materials and methods

Samples. Serum samples were collected from 378 patients with benign (atypical hyperplasia, papilloma, fibroma and ductal carcinoma) and malignant breast diseases (mean age, 45 \pm 11 years) and 70 healthy subjects (mean age, 38 \pm 9 years), all female, who were recruited from the Huai'an First People's Hospital (Huai'an, China; Table I). All patients were diagnosed by histopathology as blood samples were collected from the elbow vein of each participant. The expression of ER, PR and HER2 were analyzed if patients were suspected of having cancer. The blood was placed in a glass container, left to clot at room temperature for 1 h and stored overnight at 4°C. Serum was collected and centrifuged at 150 x g for 5 min at room temperature (to sediment erythrocytes) and then at 350 x g for 15 min at room temperature. The serum (straw-colored supernatant) was transferred to containers suitable for long-term storage and heated at 56°C for 30 min to destroy the heat-labile components of the complement.

Patients were excluded if breast cancer was present alongside other malignancies, or if they had other serious conditions, including advanced organ failure or active infections. The control group consisted of 70 healthy women. Preoperative serum samples were collected from the patients with ductal breast cancer and stored at -80°C prior to further analysis. The protocol of the present study conformed to the ethical standards of the Declaration of Helsinki and was approved by the

Ethics Committee of Huai'an First People's Hospital Faculty of Medicine. Informed consent was obtained from each patient, according to the committee's regulations.

Classification of ductal carcinoma patients. Data regarding the tumor node metastasis (TNM) classification and ER, PR, and HER2 expression in cancer tissues of the ductal carcinoma patients were acquired from the patient's medical records. The patients were classified by TNM classification and the status of ER, PR and HER2 (Table II).

Measurement of serum cytokine levels. Human IL-10 (cat. no. S1000B), IL-17A (cat. no. DY5194-05) and TGF- α (cat. no. DTGA00) ELISA kits were purchased from R&D Systems, Inc. (Minneapolis, MN, USA). Levels of serum IL-10, IL-17A and TGF- α from patients and healthy subjects were determined by ELISA following the manufacturer's protocol.

Statistical analysis. Mean \pm standard error of the mean were calculated from data. Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad, Inc., La Jolla, CA, USA). Differences among groups were evaluated using the one-way analysis of variance, followed by a post hoc multiple comparisons test (Tukey's test). Pearson's correlation coefficient was used to assess correlations between IL-10, IL-17A and TGF- α levels in patients with ductal carcinoma. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of recruited patients with mammary diseases and healthy subjects. The age difference between patients and healthy groups was compared to exclude the factor of age on the production of cytokines. There were no significant differences in the mean age of all recruited patients with mammary diseases (ductal cancer, atypical hyperplasia, papilloma, and fibroma) and healthy subjects (Table I).

Serum IL-10 levels in benign and malignant breast diseases. Serum levels of IL-10 in subjects with atypical hyperplasia and ductal carcinoma were significantly higher than those in healthy women, and in patients with papilloma or fibroma ($P < 0.01$; Fig. 1A). Patients with TNM stage II and III ductal carcinoma exhibited significantly higher serum IL-10 levels than those with stage I ductal carcinoma ($P < 0.0001$; Fig. 1B). Furthermore serum IL-10 levels were significantly higher in patients with ductal carcinoma that were ER⁻ compared with those that were ER⁺ ($P < 0.0001$; Fig. 1C). IL-10 levels were significantly higher in sera collected from patients with ductal carcinoma that were PR⁺ than in the sera of healthy controls ($P < 0.01$); however, IL-10 levels did not differ significantly between patients with ductal carcinoma that were PR⁺ and those that were PR⁻ (Fig. 1D). Furthermore, there was no significant difference between IL-10 levels in patients with ductal carcinoma that were HER2⁺ compared with those that were HER2⁻ (Fig. 1E).

Serum IL-17A levels in benign and malignant breast diseases. Only in patients with atypical hyperplasia were the serum

Table I. Demographic data of recruited patients with mammary diseases and healthy subjects.

Demographic	Ductal cancer (n=110)	Atypical hyperplasia (n=95)	Papilloma (n=85)	Fibroma (n=88)	Healthy subjects (n=70)	P-value
Mean age, years	46±19	40±16	39±13	37±17	38±9	P>0.05 ^a

^aP>0.05 in patients with benign and malignant breast diseases (ductal cancer or atypical or hyperplasia or papilloma or fibroma) vs. healthy subjects. Data are presented as the mean ± standard error of the mean.

Table II. Demographic data of patients with ductal carcinoma.

Variable	Classification	No. of patients with ductal carcinoma (n=110)
ER	Negative	55
	Positive	55
PR	Negative	50
	Positive	60
HER-2/Neu	Overexpressed	45
	Not overexpressed	65
TNM	I	25
	II	50
	III	35

TNM, tumor node metastasis; ER, estrogen receptor; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2.

IL-17A levels significantly higher than those in healthy women (P<0.05). IL-17A levels were slightly higher in patients with papilloma and fibroma compared with healthy volunteers; however, this difference was not significant (Fig. 2A). Although IL-17A levels did not differ significantly between patients with ductal carcinoma and healthy subjects, IL-17A levels in patients with stage III ductal carcinoma were significantly higher than those in healthy volunteers (P<0.01; Fig. 2B). IL-17A levels were significantly higher in patients with ductal carcinoma that were ER⁻ compared with those that were ER⁺ (P<0.0001; Fig. 2C). This was also the case in patients that were PR⁻ compared with those that were PR⁺ (P<0.05; Fig. 2D). However, IL-17A levels did not differ significantly in between patients with ductal carcinoma that were HER⁺ and those that were HER⁻ (Fig. 2E).

Serum TGF-α levels in benign and malignant breast diseases. Serum levels of TGF-α were significantly higher in patients with papilloma, fibroma and ductal carcinoma than in healthy women (P<0.05; Fig. 3A). However, there were no significant differences in levels of TGF-α between patients with atypical hyperplasia and healthy women. Patients with ductal carcinoma classified as TNM stage II also exhibited significantly higher serum TGF-α levels than those classed as TNM stage I (P<0.01; Fig. 3B); however there were no significant differences in TGF-α levels between patients with stage III ductal carcinoma and those with stage I ductal carcinoma (Fig. 3B).

TGF-α levels did not differ significantly between patients that were ER⁻ compared with those that were ER⁺ and there were no significant differences between TGF-α levels between patients that were PR⁺ and those that were PR⁻ (Fig. 3C and D). However, TGF-α levels were significantly higher in patients that were HER2⁺ compared with those that were HER2⁻ (P<0.05; Fig. 3E).

Correlations between IL-10, IL-17A, and TGF-α levels in patients with ductal carcinoma. In samples isolated from ductal carcinoma patients, a significant positive correlation was identified between TGF-α and IL-17A levels (P<0.001; Fig. 4A). However, there was no correlation between TGF-α and IL-10 levels (Fig. 4B) and no correlation between IL-17A and IL-10 levels in patients with ductal carcinoma (Fig. 4C).

Discussion

Despite wide interest in the development of novel diagnostic biomarkers for breast cancer (8,10,20), limited data have been collected regarding whether IL-10, IL-17A and TGF-α levels are associated with the clinical stage of breast cancer, as well as ER, PR and HER2 status. The results of the present study revealed that elevated serum levels of IL-10, IL-17A and TGF-α are strongly associated with ductal carcinoma and are associated with a more severe clinical stage of this disease. IL-10 and IL-17A levels were also associated with ER and PR status in ductal carcinoma. Thus, these biomarkers may be used to diagnose women with breast cancer and to identify patients with a poor prognosis that may benefit from more aggressive forms of treatment and management.

IL-10 is a pleiotropic cytokine able to suppress and stimulate the immune response (16,21). It has been suggested that patients with atypical hyperplasia may have an increased risk of tumorigenesis (22). In the current study, serum IL-10 levels in patients with atypical hyperplasia were significantly higher compared with healthy subjects and those with papilloma or fibroma. It is therefore possible that these increased IL-10 levels in patients with atypical hyperplasia may promote the development of breast cancer. The results also demonstrated that IL-10 levels were higher in sera collected from patients with stage II and III ductal carcinoma than in the sera of patients with stage I carcinoma. These results were not consistent with those reported by Ikeguchi *et al* (19), who reported that serum IL-10 levels were not correlated with tumor stage in patients with gastric cancer. Therefore, it was speculated that the intensity of the immune response varies among patients with different types of cancer. For example, the antigens of

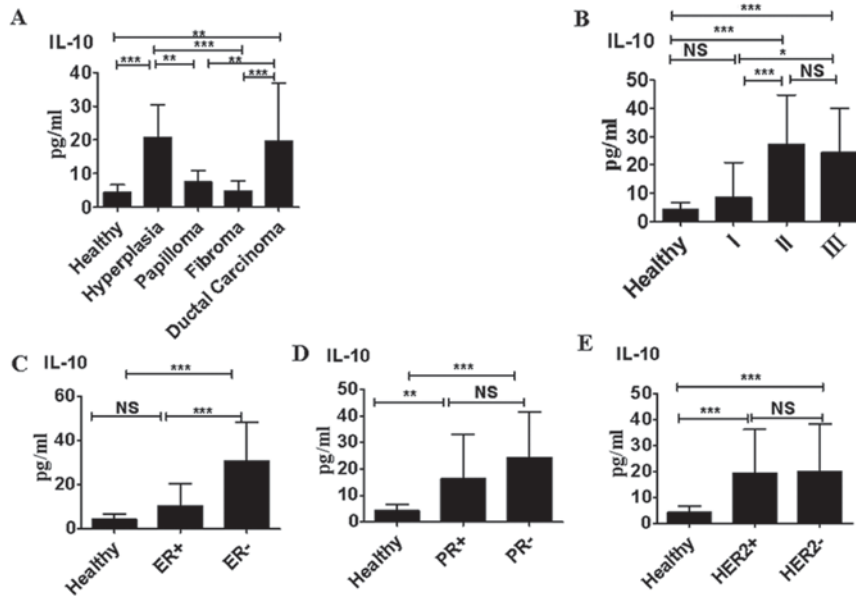


Figure 1. Serum IL-10 levels in benign and malignant breast diseases. (A) IL-10 levels in healthy women (n=70) and patients with atypical hyperplasia (n=95), papilloma (n=85), fibroma (n=88) or ductal carcinoma (n=110). (B) Serum IL-10 levels in patients with different clinical stages of ductal carcinoma. (C) Serum IL-10 levels in patients with ductal carcinoma that were ER⁺ compared with those that were ER⁻. (D) Serum IL-10 levels in patients with ductal carcinoma that were PR⁺ compared with those that were PR⁻. (E) Serum IL-10 levels in patients with ductal carcinoma that were HER2⁺ positive compared with those that were HER2⁻. *P<0.05; **P<0.01 and ***P<0.0001. Data are representative of three different experiments and presented as the mean ± standard error of the mean. IL, interleukin; NS, not significant; ER, estrogen receptor; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2.

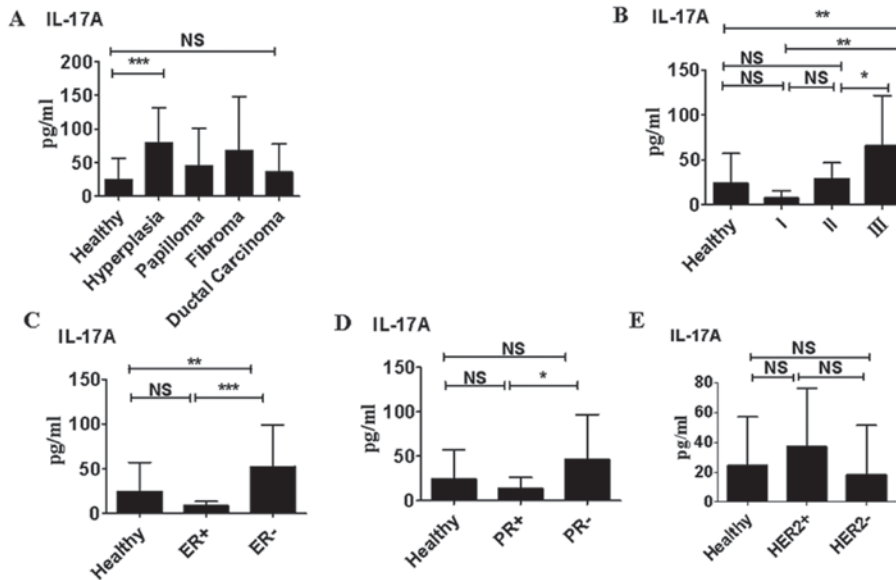


Figure 2. Serum IL-17A levels in benign and malignant breast diseases. (A) In healthy women (n=70) and patients with atypical hyperplasia (n=95), papilloma (n=85), fibroma (n=88) or ductal carcinoma (n=110). (B) By clinical tumor stage in patients with ductal carcinoma. (C) In patients that were ER⁺ vs. those that were ER⁻. (D) Patients who were PR⁺ vs. those that were PR⁻. (E) In patients that were HER2⁺ vs. those that were HER2⁻. *P<0.05; **P<0.01 and ***P<0.0001. Data are representative of three different experiments and presented as the mean ± standard error of the mean. IL, interleukin; ER, estrogen receptor; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2; NS, not significant.

ER and HER2 are highly expressed on breast cancer cells but not gastric cancer cells, and these antigens may promote or suppress cytokine production (23). The results of the current study indicated that high serum IL-10 levels were associated with negative ER expression, but not with PR and HER2 expression.

IL-17A-producing TILs are found in greater numbers in breast tumor tissue than in healthy mammary tissue (9,12). The

results of the current study revealed that serum IL-17A levels did not differ significantly between healthy women and patients with ductal carcinoma; however, IL-17A levels in patients with stage III ductal carcinoma were significantly higher than those in patients with stages I and II ductal carcinoma and healthy volunteers. This suggests that IL-17A serves a critical role in promoting the progression of cancer (9,14) The results of the current study demonstrated that serum IL-10 and IL-17A levels

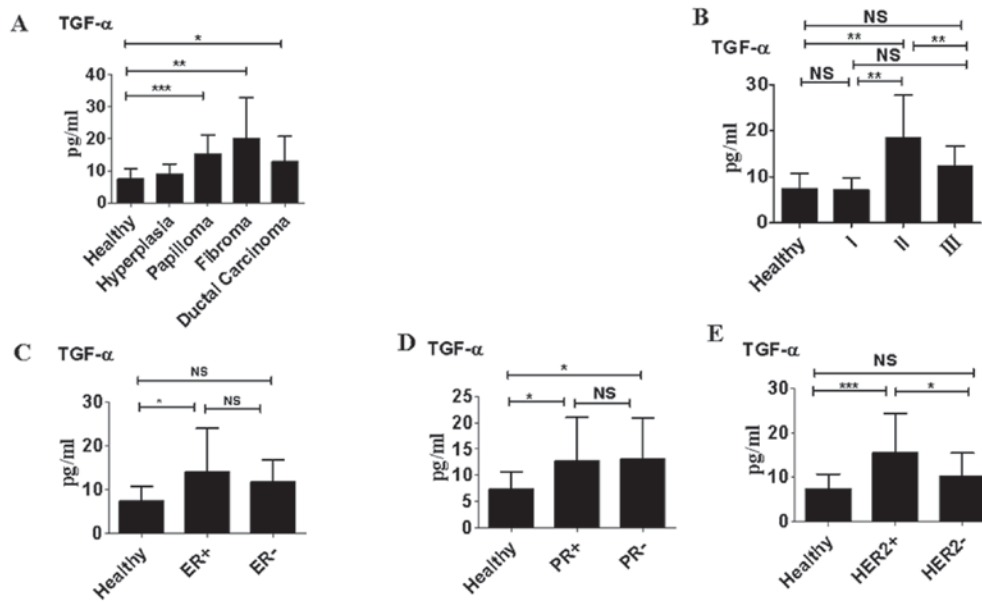


Figure 3. Serum TGF- α levels in benign and malignant breast diseases (A) In healthy women (n=70) and patients with atypical hyperplasia (n=95), papilloma (n=85), fibroma (n=88) or ductal carcinoma (n=110). (B) By clinical tumor stage in patients with ductal carcinoma. (C) In patients that were ER⁺ vs. those that were ER⁻. (D) In patients that were PR⁺ vs. those that were PR⁻. (E) In patients that were HER2⁺ vs. those that were HER2⁻. *P<0.05; **P<0.01; ***P<0.0001. Data are representative of three different experiments and presented as the mean \pm standard error of the mean. TGF, transforming growth factor α ; ER, estrogen receptor; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2; NS, not significant.

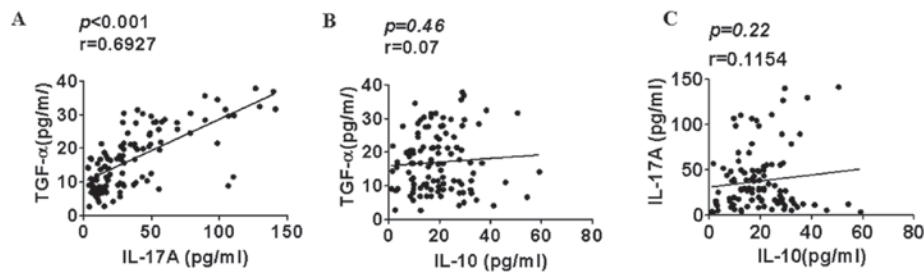


Figure 4. Correlation between levels of the cytokines IL-10, IL-17A and TGF- α in patients with ductal carcinoma. (A) Correlation between IL-17A and TGF- α levels. (B) Correlation between TGF- α and IL-10 levels. (C) Correlation between IL-10 and IL-17A levels. IL, interleukin; TGF- α , transforming growth factor α .

are increased in patients with atypical hyperplasia. These results may not actually be contradictory, as IL-10 and IL-17A may promote tumorigenesis. Furthermore, in the current study, serum IL-17A levels were associated with the absence of ER in tumor tissue. Specifically, IL-17A levels were significantly elevated in patients that were ER⁻ and PR⁻. The mechanisms underlying this association between IL-17A and ER remain unknown. One possibility is that ER deficiency in breast cancer may directly promote the expansion of IL-17A-producing TILs (9).

In breast cancer, TGF- α may promote the growth and progression of tumors via an autocrine/paracrine loop involving the epidermal growth factor receptor (10,13,24). In the current study, serum TGF- α levels in patients with papilloma, fibroma and ductal carcinoma were significantly higher than those in healthy subjects; this suggests that TGF- α may also promote tumor development. High serum TGF- α levels were also associated with a more severe tumor stage and were significantly increased in patients that were HER2⁺; however there was no association between serum

TGF- α levels and ER or PR expression. Since mammary gland epithelial cells are able to secrete TGF- α , it was hypothesized that HER2 expression in tumor cells may enhance TGF- α production.

It was then assessed whether there were correlations between serum IL-17A and TGF- α , IL-10 and TGF- α , and between IL-10 and IL-17A. The results demonstrated that IL-10 levels increased as IL-17A levels increased; however, there appeared to be no significant association between IL-10 and IL-17/TGF- α levels. However, a strong positive correlation between TGF- α and IL-17A was identified. Therefore, TGF- α and IL-17A may function synergistically during the initiation and development of tumors.

In conclusion, the results of the present study suggest that increased serum levels of IL-10, IL-17 and TGF- α are associated with ductal carcinoma. Increased levels of these cytokines are also associated with a more severe clinical stage of ductal carcinoma, and with the negative expression of ER and HER2. Thus, these cytokines may be developed as potential diagnostic and prognostic cancer biomarkers.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZL and ML performed the immunoassays. JS collected the patient and control samples. DX performed the data analysis. YM drafted the manuscript. YM and YJ designed the study and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The protocol of the present study conformed to the ethical standards of the Declaration of Helsinki. All procedures were approved by the Ethics Committee of Huai'an First People's Hospital Faculty of Medicine (Huai'an, China). Informed consent was obtained from each patient, according to the committee's regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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