Original Article

Increased Transforming Growth Factor-β and Interleukin-17 Transcripts in Peripheral Blood of Breast Cancer Patients with Different Clinical Stages

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Abstract

Background: Currently, cancer as major problem around the world threatens human health and has a high incidence in developing countries. Many reports have showed that there is a dysfunction in the immune system of cancer patients. Interleukin-17 (IL-17) and transforming growth factor-β (TGF-β) are increased in the generated through cancers, and IL-17 is considered as a pro-inflammatory cytokine; IL-17 is an angiogenic factor and promotes tumor growth. **Materials and Methods:** In this study TGF-β and IL-17, mRNA gene expression has been measured in peripheral blood of 35 breast cancer patients and 35 normal age-matched women using quantitative real-time polymerase chain reaction method with master mix reaction containing SYBER Green. β-actin gene was used as housekeeping gene. **Results:** Our data demonstrated a significant upregulation of TGF-β and IL-17 gene expression ($P < 0.05$) in patient's peripheral blood compared to normal healthy control. Levels of genes expression were higher in the breast cancer group compared to the control group. **Conclusion:** TGF-β and IL-17 expression could be used as a diagnostic marker for detecting breast cancer.

Keywords: Breast cancer, gene expression, interleukin-17, real-time polymerase chain reaction, transforming growth factor-β

Introduction

Breast cancer is one of the most often diagnosed cancers and the leading reason of cancer death in females worldwide, comprising 23% of the total new cancer cases and 14% of the total cancer death in 2008; worldwide, breast cancer is the fifth most common cause of cancer mortality.[1] In Iraq, breast cancer is one of the greatest significant contributors to female malignancy, accounting for about one-third of the registered female cancers rendering to the latest Iraqi cancer registry,[2] and is the second reason of cancer-associated deaths.[3] Recently, a subset of T-helper (Th) lymphocytes secreting mainly the pro-inflammatory interleukin-17 (IL-17) cytokines, the Th17 cells, has gained considerable attention, assumed their influence to infectious, auto, and cancer immunity, consequently, the IL-17 pro-inflammatory which has many functions.

IL-17 has been shown to enhance the production of IL-6 and IL-8, and the expression of intracellular adhesion molecule-1 in fibroblasts, macrophages, and nitric oxide in human osteoarthritic cartilage. Since inflammation is also tightly correlated to cancer development, these cytokines have been also intensively investigated in the context of cancer development and progression. Recent research provided substantial insights into the mode of action of Th17 and IL-17 cytokines in a variety of tumors. Tartour *et al.*[4] reported that IL-17 was expressed in cervical cancer in four of six patients, and they also demonstrated that transduction of cDNA encoding IL-17 promoted tumorigenicity of human cervical tumors in nude mice. The role of these cytokines in breast cancer pathogenesis is not fully understood. In this study report, the expression of IL-17 and transforming growth factor-β (TGF-β) mRNA in peripheral blood mononuclear cells (PBMCs) of patients with breast cancer compared the results according to the patients' clinical or pathological status.

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Materials and Methods

Study subjects

Only 35 women with breast cancer participated in this study, all patients agreed to contribute in our study and signed the informed consent form. The patients were referred to the specialist center for oncology and blood disease at AL-Sadr teaching hospital in Basra city and were randomly selected during the period March 2016–2017.

Peripheral blood samples (2 ml) with EDTA were collected. Patient's data including age, histologic diagnosis, tumor grade, clinical stage, tumor-node-metastasis (TNM), estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 were documented. Thirty-five healthy controls were also selected among individuals who were a colleague staff and had no clinical evidence and history of malignancy or autoimmune disorders.

RNA isolation and reverse transcription

Total RNA was extracted from blood after lysis of the red blood with GENEzol™ reagent rendering to the manufacturer's instructions. The quantity and quality of the extracted RNA were valued by agarose gel electrophoresis, and the total RNA concentration was estimated by spectrometry at 260 nm. To synthesize cDNA, we used 5 μg of total RNA and QuantiTect Reverse Transcription Kit (Bioneer, Korea).

Quantitative real-time polymerase chain reaction

The quantities and expression of TGF-β and IL-17 gene transcripts were determined with a Bioneer for quantitative real-time polymerase chain reaction (qRT-PCR) and SYBER Green PCR Master Mix. Expression of β-actin housekeeping gene was used to normalize the level of target gene expression. Each PCR reaction was carried out in a final volume of 20 μl that contained 10 μl of the cDNA product, 1 pmol of each primer, 2 μl DEPEC-distilled water, and 10 μl 2X GreenStar Master Mixes. Thermal cycling for all genes was done with denaturation step at 95°C for 15 min, following by 45 cycles (denaturation at 95°C for 30 s, annealing Tm-5 for 1 min, and extension at 72°C for 7 min). The qRT-PCR amplification products were examined by melting curve analysis and 1% agarose gel electrophoresis. Table 1 shows the forward and reverse primers for β-actin, TGF-β, and IL-17.

Statistical analysis

The amounts of TGF-β and IL-17 gene transcripts in peripheral blood were compared to the corresponding values from control samples by one-way ANOVA using SPSS statistics program version 22 (IBM, Armonk, NY, United states of America), and $P \leq 0.05$ was used as the level of significance.

Results

Clinical and pathological characteristics

Data on age, tumor histology type, clinical stage, histological grade, lymph node, distant metastasis, ER receptor, PR, and human epidermal growth factor-2 were achieved from all of 35 patients. Table 2 shows the distribution of patients according to different clinical criteria.

Interleukin-17 gene expression

The findings implied that expression of IL-17 transcript in PBMC in different grades of breast cancer patients was significantly higher than healthy controls [$P < 0.05$, Figure 1]. No association was found between the level of IL-17 gene expression and clinicopathological characteristics [Tables 3 and 4].

Transforming growth factor-β gene expression

The result of the study provided evidence that the expression of TGF-β gene in patients with breast cancer was increased

Table 1: Forward and reverse primers of β-actin, interleukin-17, and transforming growth factor-β genes for real-time polymerase chain reaction amplification

Primer	Sequence
β-actin forward	ACAGAGCCTCGCCTTTGCCG
β-actin reverse	CACCATCACGCCCTGGTGCC
IL-17 forward	GGACTGTGATGGTCAACCTG
IL-17 reverse	CTCCCAGATCACAGAGGGAT
TGF-β forward	TGGTTGAGCCGTGGAGGGGA
TGF-β reverse	CTCGGCGGCCGGTAGTGAAG

IL-17: Interleukin-17, TGF-β: Transforming growth factor-β

Table 2: Patient distribution according to different clinical criteria

Pathological characteristic	Frequency ($n=35$)
Grade	
G1	3
G2	17
G3	15
Stage	
T1	3
T2	21
T3	9
T4	2
Metastasis	
Positive	14
Negative	21
LN	
Positive	22
Negative	13
ER	
Positive	23
Negative	12
PR	
Positive	23
Negative	12
HER-2	
Positive	11
Negative	24

ER: Estrogen receptor, HER-2: Human epidermal growth factor receptor-2, PR: Progesterone receptor, G1: Grade 1, G2: Grade 2, G3: Grade 3, T1: Stage 1, T2: Stage 2, T3: Stage 3, T4: Stage 4, LN: Lymph node

compared to the control group but no significant [Figure 2]. Nevertheless, the relative expression of TGF-β was about 23-fold higher in patients' comparison with control. However, there was no significant association between TGF-β expression and any of the clinical and pathological characteristics [Tables 3 and 4].

Table 3: Association between clinical factors and genes expression in breast cancer patients

Variables	TGF-β expression	IL-17 expression
ER		
Positive	0.1	0.2
Negative	0.7	0.03
PR		
Positive	0.1	0.2
Negative	0.7	0.03
HER-2		
Positive	0.6	0.1
Negative	0.07	0.3
LN		
Positive	0.4	0.1
Negative	0.03	0.3
Metastasis		
Positive	0.05	0.2
Negative	0.4	0.1

ER: Estrogen receptor, HER-2: Human epidermal growth factor receptor 2, PR: Progesterone receptor, LN: Lymph node, IL-17: Interleukin-17, TGF-β: Transforming growth factor-β

Table 4: Association between stage and grade and genes expression in breast cancer patients

Variables	TGF-β expression	IL-17 expression
G1	0.003	0.13
G2	0.2	0.2
G3	0.2	0.1
T1	0.01	0.01
T2	0.4	0.04
T3	0.04	3.2
T4	0.2	0.12

G1: Grade 1, G2: Grade 2, G3: Grade 3, T1: Stage 1, T2: Stage 2, T3: Stage 3, T4: Stage 4, IL-17: Interleukin-17, TGF-β: Transforming growth factor-β

Discussion

TGF-β and IL-17 expression was affected by breast cancer infections which are demonstrated in this study. There were increased in the expression of IL-17 and TGF-β genes in patient comparing with control group. It has extended been consent that IL-17 and TGF-β play a remarkable role in the pathogenesis of breast cancer, but the accurate mechanisms are not well characterized, and both suppressing and promoting tumor functions have been described. There are different interconnected processes such as tumorigenesis, tumor proliferation, tumor angiogenesis/angiostasis, tumor metastasis, and chemoresistance in which IL-17 may have a central role.[5]

Interestingly, the studies were revealed that the key factor stimulating the generation of Th17 cells from naïve T-cells in humans is TGF-β that lengthways with certain inflammatory cytokines (either TGF-β with IL-21 or TGF-β with IL-6 and IL-23) and persuades the transcription factor (RoRgammat).[6] The initiation of RoRgammat leads then to the development of naïve T-cells to Th17 cells which produce IL-17.[7]

The expression of IL-17 mRNA was investigated by Zhang *et al*.[8] who noticed increased expression of IL-17 and IL-23 mRNA in tumor tissues from patients with gastric cancer, and the author also established that IL-17 expression in breast cancer tissue is mostly restricted to macrophage, and IL-17 released by macrophage has a possible role in promoting tumor progression and invasion. Miyahara *et al*.[9] reported that IL-17-positive T-cells are significantly increased in cancer tissue, peripheral blood, bone marrow, and spleens of patients with various cancers or in tumor-bearing mice. Kato *et al*.[10] reported the high expression of IL-17 mRNA

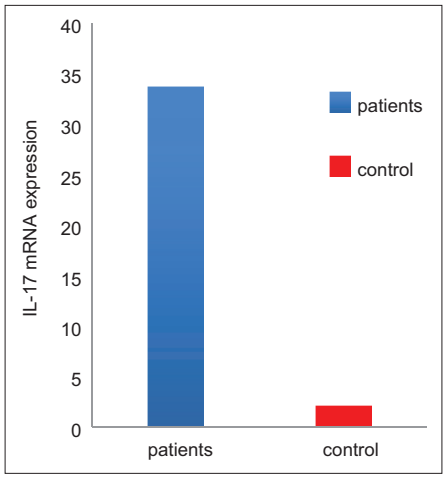

Figure 1: Effect of breast cancer on the mRNA expression of interleukin-17. A significant difference at $P < 0.05$

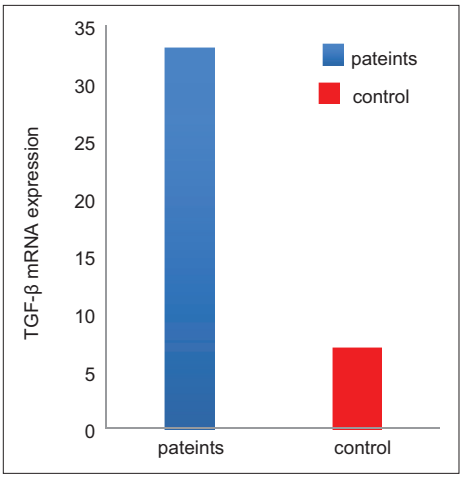

Figure 2: Effect of breast cancer on the mRNA expression transforming growth factor-β. A significant difference at $P < 0.05$

in ovarian cancer, with a significant role in tumor growth and angiogenesis.

The above studies document evidence that IL-17 is a multifunctional cytokine have the ability to promote growth and expansion. Intratumoral IL-17 is able to induce the production of vascular endothelial growth factor-C (VEGF-C) and VEGF-D. In addition, document evidence that Th17 cells also produce IL-21, which in an autocrine manner inhibits forkhead box P3 (FoxP3) and IFN-γ expression and further supports the Th17 cell phenotype. Th1 or Th2 cell-inducing cytokines (IFN-γ, IL-4) rather inhibit differentiation toward Th17 cells. The effect of TGF-β has been shown to depend on its local concentration while low levels of TGF-β facilitate Th17 differentiation; high TGF-β levels induce the expression of the Treg lineage-specific transcription factor FoxP3. FoxP3 inhibits the transcriptional activity of RORyt, leading to differentiation toward Tregs. IL-6 abrogates this inhibition and induces Th17 differentiation.

The expression of IL-17 has been demonstrated in various types of tumors including lymphoma, myeloma, breast cancer, colon cancer, gastric cancer, hepatocellular cancer, melanoma, ovarian cancer, pancreatic cancer, and prostate cancer. The results of this study also indicated that there was no significant correlation which was found between the expression of IL-17 and any clinic pathological features such as stage, histological type, grade, lymph node metastasis, or survival; this is agree with Zou and Restifo[11] who reported that the number of IL-17A-producing cells correlates with poor survival in patients with hepatocellular carcinoma, whereas the number was decreased in patients with advanced ovarian cancer, lung cancer, or non-Hodgkin's lymphoma. So thus, Th17 cells and IL-17A may play different roles depending on the tumor type and stage. Recent studies highlighted association between increased expression of cancer-infiltrating TH17 cells and high risk for several types of cancer. For example, Steiner *et al*.[12] demonstrated that the expression of IL-17 mRNA was increased in ovarian cancer tissue.

TGF-β is one of the most abundant growth factors, which has a dual role in tumor, acting as a tumor suppressor in early stages of carcinogenesis and a pro-oncogenic role in the last steps of the metastases. TGF-β has emerged as critical roles in the epithelial-to-mesenchymal transition; however, their participation is not only limited to their role but also increases angiogenesis and induces immunosuppression.[13] Expression of the TGF-β gene transcript in PBMC was increased 23-fold in patients compared to control, such elevations are probably due to tamoxifen treatment, and tamoxifen is currently the most frequently used endocrine therapy for ER-positive breast cancer patients. We have shown previously that the action of tamoxifen is at least partially mediated through activation of TGF-β.

Other studied features of TGF-β, that it is the most important immunosuppressive cytokine referred to escape the immune system surveillance and to induce tumor growth and Metastasis.[14] This step was done through cancer cells elevated levels of TGF-β, which acts on nontransformed cells present in tumor microenvironment as well as distant cells in the host. This way suppresses antitumor immune response and creates an environment of immune tolerance. Moreover, TGF-β also acts as a chemoattractant for monocytes and macrophages, which then migrate toward the tumor. In addition, macrophages modified in tumor microenvironment further support tumor invasion, angiogenesis, and metastasis and also lead to diminished antigen presentation.[15] TGF-β is potentially able to stimulate reactive oxygen-specific (ROS) production. ROS is a family of reactive molecules which may contribute to tumorigenesis. However, conflicting data exist about the influence of TGF-β on the development and progression of breast cancer.

Buck *et al*.[16] found that the expression of TGFII is correlated with poor prognosis and represents an independent prognostic marker when analyzed the correlation of TGRI and TGRII expression with overall survival in breast cancer patients and discriminate between hormone dependent and independent. Sheen-Chen *et al*.[17] pointed out that the relationship between TGF-β and breast cancer seems to be based on the stage of cancer, and more specifically women with more advanced lymph node status, TNM staging, and poorer histologic grade have higher TGF-β1 serum levels. Teng *et al*.[18] demonstrated that TGF-β production by tumor cells can convert effector T-cells into Treg which, in turn, suppress other tumor-infiltrating effector T-cells. A decreased expression of TβRs is considered to be one of the possible mechanisms responsible for the loss of TGFβ sensitivity and the enhanced tumor progression in many types of cancer. However, the relationship between TGF-β expression and prognosis is controversial in cancer. Furthermore, Hamidinia *et al*.[19] demonstrated a significant upregulation of IL-10, TGF-β, P35, EB13, and foxp3 gene expression in peripheral blood of breast cancer patients.

In conclusion, the results of our study indicate that the expression of IL-17 and TGF-β is increased. This finding may reflect a vigorous pro-inflammatory reaction orchestrated by the host immune system against cancer.

Conclusion

The result of this exploratory study suggests that there is suppression in the immune system of breast cancer through TGF-β, which may be due to stimulation of T-regulatory cells. In addition, our results are showed that IL-17 affects prognosis.

The results of our study indicate that the expression of IL-17 and TGF-β is increased. This finding may reflect a vigorous pro-inflammatory reaction orchestrated by the host immune system against cancer.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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