Oncology
and Radiotherapy ©
17(9) 2023: 424-427 • REVIEW ARTICLE

Association of TGF-B1 gene expression with breast cancer risk in a sample of Iraqi women

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ABSTRACT

Breast carcinoma is the most prevalent cancer-related cause of death in women, and metastasis is the main factor in morbidity. The total number of new cases of cancer in Iraq during the year 2019 was 35,864.

TGF (Transforming Growth Factor) is a multifunctional cytokine whose abnormal expression is linked to cancer development and metastasis. The tumor growth factor TGF-β is a major component of Epithelial–Mesenchymal Transition (EMT), immune evasion, and metastasis during cancer progression. It is produced by a variety of cells within the Tumor Microenvironment (TME), and it is responsible for regulating the activity of cells in this milieu. TGF-b is also central to immune suppression within the tumor microenvironment, and recent studies have revealed roles in tumor immune evasion and poor responses to cancer immunotherapy. Then, focusing on cancer, we discuss the roles of TGF-b signaling in distinct immune cell types and how this knowledge is being leveraged to unleash the immune system against the tumor.

Key words: breast cancer, TGF-B1, cytokines, EMT, TME

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Word count: 2418 **Tables:** 01 **Figures:** 00 **References:** 29

Received:- 22 August, 2023, Manuscript No.: OAR-23-111152

Editor Assigned: 26August, 2023, PreQC No.: OAR-23-111152 (Q)

Reviewed: 04 September2023, QC No.: OAR-23-111152 (PQ)

Revised: 12 Septembe,2023, Manuscript OAR-23-111152(R)

Published: 19 September 2023, Invoice No. J-111152

INTRODUCTION

Cancer is the major reason for morbidity and mortality worldwide. In 2020, a global cancer burden approximated rate to 19.3 million patients diagnosed with cancer [1]. In Iraq, the total number of new cases of cancer during the year 2019 was 35,864 with an incidence of 91.66/100,000 P, While the rate that was recorded in 2010 was 18,482 with an incidence of 56.89/100,000 P [2]. Cancer can simply be defined as a class of diseases or disorders that is characterized by an uncontrolled division of cells and the ability of these abnormal cells to spread, either by direct growth into adjacent tissues through invasion or by implantation into distant sites by metastasis (where cancer cells are transported through the bloodstream or lymphatic system) [3]. Breast Cancer (BC) is one of the most common malignant tumors and the second leading cause of cancer in women. Approximately, 1.5 million new cases are annually diagnosed with breast cancer and almost 460,000 patients died each year due to BC chemo-resistance and metastasis [4]. Approximately, 7,109 new cases are diagnosed with breast cancer in 2019 [2]. Breast Cancer biological characteristics are routinely used for early detection, prognosis, and selection of the therapeutic strategy, including histologic subtype, grade, lymph node status, hormone receptor, and Human Epidermal Growth Factor Receptor 2 (HER2) statuses [5]. Some of the mentioned characteristics are related to patients' survival and post treatment clinical outcomes [6]. However, several BC patients, who had similar characteristics, showed different clinical outcomes. Therefore, biological features have limitations with regard to diagnosis, prognosis, and clinical outcomes' prediction [7]. Thus, there is still need to develop a cost-effective and accurate screening method for this cancer and discover new biomarkers to improve diagnosis, prognosis and prediction [8], and novel diagnostic and prognostic approaches are urgently required for the identification of new personalized therapeutic methods that improve BC patients' quality of life.

TGF-β exists as three isoforms, TGF-β1, TGF-β2, and TGF-β3, which are encoded by distinct genes. They belong to the TGF-β superfamily of proteins, which contains over 30 members including activins, Bone Morphogenetic Proteins (BMPs), and growth and differentiation factors. TGF-β superfamily members have diverse functions in development, homeostasis, repair and disease, which signal through canonical (Smad signaling) and non-canonical signaling pathways (24–26) TGF-b receptors. The Smad signaling pathway includes two distinct pathways 1) the TGF-β-Smad pathway, which is mediated via Smad 2 and Smad 3 phosphorylation, and 2) BMP-Smad pathway which involves Smad 1/5/8 phosphorylation Both signaling pathways are critical

Tab. 1. Comparison between TGF-B1 expression between control and breast cancer patients.

Fold TGF-B1 patients	3.2972
Fold TGF-B1 controls	1.1242
P.VALUE	**0.0001**

for normal alveolar and pulmonary vasculature development and have been implicated in the pathogenesis of BPD [9,10]. Epithelial-Mesenchymal Transition (EMT) is an extremely important basic procedure in the process of normal embryonic development, wound healing and malignant epithelial tumorigenesis. It can alter epithelial cells morphology and promote the transition of these cells into motile mesenchymal cells. Consequently, these changes can damage cell-cell and cell extracellular matrix junctions, so that cells can migrate to other parts of the body [11].

TGF signaling is involved in many of the cellular processes that are involved in the development and progression of cancer. The regulation of Cancer Stem Cell (CSC) homeostasis, inhibition of immune response, activation of Epithelial-Mesenchymal Transition (EMT) and metastasis are all under the control of TGF signaling [12].

MATERIALS AND METHODS

Fifty Iraqi Women patients with breast cancer who attended Al-Amal hospital and Al-Andalus specialist hospital during the period extended from 1 December 2021 to the 23 of February 2022 with age ranged from 30 years – 67 years were registered in this study.

The required information about the patients and the histopathological properties of the tumors recorded from the patients' files.

All of the patients were diagnosed. These patients were from different stages of the disease, different age groups (30 years – 67 years).

All the cases subjected to molecular study. The samples preservation with TRIzol.

RNA extraction

RNA was isolated from serum samples according to the protocol of TRIzol™ Reagent. 0.2 mL of chloroform was added to each tube. All mixes were Incubated for 2 minutes – 3 minutes then centrifuged for 10 minutes, the mixture was separated into a three phase. The aqueous phase containing the RNA was transferred to a new tube. 0.5 mL of isopropanol was added to the aqueous phase and incubated for 10 minutes then centrifuged for 10 minutes, Total RNA was precipitated. For each tube, 0.5 mL of 70% ethanol was added and vortexed briefly then centrifuged for 5 minutes, Ethanol then aspirated and air-dried the pellet. Pellet was rehydrated in 50 µl of Nuclease Free Water.

Detection of mRNA by RT-qPCR

Total RNA containing mRNA was the starting material in RT-PCR reaction which was performed in two steps. The mRNA gene TGF-B1 expression was done by using specific primers.

First step:

To test the expression of PCR target genes, the first method was reverse ProtoScript® First Strand cDNA Synthesis Kit(NEB) company involves the conversion of RNA to cDNA. Total RNA containing mRNA was used as row material for reverse-transcription reaction.

All RNA was converted into cDNA, in RT-qPCR need primers such as mRNA using oligo-dT primers they were reverse transcribed into cDNA, and the miRNA should have specific primers for conversion of RNA to cDNA. First reaction, the total RNA and mRNA primers were added to PCR tube microfuge.

Second step:

Choosing the cDNA sample from patient and control at the same run, one tube for each gene TGFB-1 and TBP gene which is consider as a house keeping gene in this study. The detection of quantity based on fluorescent power of SyberGreen.

RESULTS

Fifty breast cancer cases and 25 healthy controls were included in the analysis. Initially, we examined the expression of TGF-B1 expression in breast cancer whole blood using Rt-qPCR.

The finding that the gene expression of TGF-B1 in breast cancer patients was greatly increased compared to that in normal individuals is interesting and suggests that TGF-B1 may play a significant role in breast cancer development and progression.

That showed significantly TGF-B1 expression (upregulation) when compared to control group ($p<0.0001$) that show in Table 1.

Breast cancer can be classified according to tumor size, the number of lymph nodes that have the tumor, and if the breast was metastatic or not.

According to age group that the gene expression study on Iraqi women patients showed that the highest percentages of breast cancer cases occurred in the age groups of 40 years - 49 years and 50 years - 59 years, indicating that these age groups may be more susceptible to developing breast cancer. However, it is important to note that there was no significant associations found between high TGF-β1 expression levels and age.

This corresponds with other demographic studies that also found no significant differences in TGF-β1 expression levels across age groups [13-15].

DISCUSSION

The result presented by this study agreed that shows there are statically significant increase of gene expression in patients to control and the up regulation of TGF-B1 promoted the migration, invasion and metastasis of breast cancer cells [16-18].

We believe This difference came in expression because the TGF-B1 is responsible for Migration and invasion through promoting Epithelial--Mesenchymal Transition (EMT) The latter leads to metastasis and chemotherapy resistance also TGF-b inhibits NK cell and neutrophil effector functions and thereby contributes to a permissive microenvironment for tumor progression and the reason of upregulation of TGF-B1 was the cell when convert

to cancer cells and after the cancer progression the change in cells lead to change in TGF-B1 signaling pathway the produces overexpression of TGF-B1 [19-22].

Also the study was showed the highest percentage was age between the (40 years - 60 years) and the second percentage was above the 60 years old and the lowest percentage age was under 40 years [23-26].

Also the gene expression of TGF-B1was increase in age group (50 years - 60 years) because women in their 50s are often experiencing hormonal changes, such as menopause, which can cause an increase in breast cancer risk. Additionally, certain genetic mutations, such as BRCA1 and BRCA2, can increase the risk of breast cancer. Lifestyle factors, such as obesity, lack of physical activity, and alcohol consumption, may also contribute to the development of breast cancer. Finally, exposure to radiation, such as radiation therapy for other medical conditions, may also increase the risk of developing breast cancer [27-29].

CONCLUSION

Our observations from this study found that TGF-B1 gene expression increase in female with breast cancer. The results show that patients with breast cancer have a higher expression of TGF-B1.

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