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Association of TGF-B1 Protein Level 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 aim of the study level of TGF-B1 between the age groups. The total number of new cases of cancer in Iraq during the year 2019 was 35,864. Transforming growth factor (TGF) 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. The aim of this study to investigate the role of TGF-B1 in Breast Cancer risk Method: measure the protein level by ELISA Sandwich method. It was concluded that found significant increase in cancer patients compare with controls, according to age group that was no- significant association and the result show significant increase in metastasis patients compare with non-metastasis group.

Keywords: Breast cancer, TGF-B1, cytokines, EMT, TME.

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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 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). Approximately1.5 million new cases of Breast cancer (BC) are annually diagnosed with and almost 460,000 patients died each year due to BC chemo resistance and metastasis (4).

Biological characteristics of Breast Cancer 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 (6). Some of the mentioned characteristics are related to patients' survival and post treatment clinical outcomes (7). 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 (8). 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 and novel diagnostic and prognostic approaches are urgently required for the identification of new personalized therapeutic methods that improve BC patients' quality of life (9).

TGFβ is a cytokine which acts as a tumor suppressor in poorly aggressive tumors while promoting epithelial to mesenchymal transition (EMT) and stimulating tumor growth and metastases in highly aggressive cancers. In breast cancer, the TGFβ that have three isomer and most important one in cancer wasTGF-B1 (10).

Tumors overproduce TGF to establish an immunosuppressive environment necessary for tumor cell survival and proliferation in advanced stages of malignancy. Cancer progression is caused by dysregulation of TGF signaling. TGF is commonly seen in high concentrations in the blood of cancer patients in advanced stages, and its role frequently alters throughout cancer progression (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).

The TGF-b system is a typical membrane-to-nucleus signaling pathway that involves direct receptor-mediated SMAD activation. Activated SMAD proteins engage to numerous loci throughout the genome as directed by partner transcription factors, the availability of which in a given cellular setting affects the cell's response (13).

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 (14).

Material and method

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 30-67 years were registers 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 was diagnosed by Oncologist and Radiologist . These patients were from different stages of the disease, different age groups (30 – 67 years). Take 2ml of blood sample in EDTA tube then centrifuge to separate the serum after that preservation with TRIzol . Twenty-Five - apparently healthy volunteers

with age ranged from 26 – 63 years, all twenty-five women were unrelated with no family history of breast cancer.

Detection the level of TGF-B1 in the serum by ELISA

In this study use Sandwich ElISA Kit (Bioassay Technology Laboratory-china) According to following steps:

1. Serial Standard Reconstitute the 120µl of the standard (4800ng/L) with 120µl of standard diluent to generate a 2400ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (2400ng/L) 1:2 with standard diluent to produce 1200ng/L, 600ng/L, 300ng/L and 150ng/L solutions. Standard diluent serves as the zero standard (0 ng/L), Dilution of standard.
2. Fifty microliter of buffer was added in the first whole then put the serial diluent from zero to reach 2400 ng/L.
3. Forty microliter that was added from each sample in the same wells plate.
4. A conjugated reagent can be prepared by mixing an antibody with streptavidin-HRP.
5. Fifty microliters from conjugated was added to each well in the sample plate then leave for an hour for reaction at room temperature.
6. Wash step: 20ml of wash buffer(25x) was mix with 500ml distilled water then the plate was washed twice to remove nonbinding antigen and antibody after that was leaved to dry.
7. TMB mix prepared by mix 6 ml of solution A with 6 ml of solution B then added 50µl of TMB to all wall in the plate after that directly to dark room for 10 mint.
8. Fifty microliters from stop solution was added to all wholes in the plate to end the reaction the colored was change to yellow.
9. Quantity was be measure be wavelength 450 nm.

Results and discussion

Fifty breast cancer cases and 25 healthy controls were included in the analysis. initially, was examined the protein level of TGF-B1 in serum.

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.

BC group showed significantly increase of TGF-B1 level in serum, when compared to control group ($p<0.0001$) as show in (Table 1).

In a similar study TGF-B1 protein level was significantly increase in patient's comparison with healthy controls the high level of TGF-B1 promoted the migration, invasion and metastasis of breast cancer cells (15).

The result presented by this study agreed with(16) that shows there are statically significant increase of protein level in patients compare to control and the up regulation of TGF-B1 in breast cancer in patients contribute in the tumor progression because the TGF-B1 is responsible for Migration and invasion through promoting epithelial-to-mesenchymal transition (EMT) The latter leads to metastasis and chemotherapy resistance also TGF-b inhibits Natural Killer cells and neutrophil effector functions and thereby contribute to a permissive microenvironment for tumor progression and the reason of upregulation after the cancer progression the change in cells lead to change in TGF-B1 signaling pathway the produces overexpression of TGF-B1, these results agreed with the results represented by (17).

Table (1): Comparison between the average of TGF-B1 level between control and breast cancer patients

Group	TGFB1 (mean +SE)
PROTEIN LEVEL OF Patients	Mean 231.54 ng/ml
PROTEIN LEVEL OF Control	Mean 87.85ng/ml
P.VAULE	0.0001

Groups level of protein according to age

According to age group the patients divided four groups the high percentage of percentage of breast cancer occurred between 50-59 (44%) and the lowest percentage of patients was (30-39), which indicated that breast cancer is more frequent in high age groups than others groups this result enforces the fact that the change in the hormonal factors in female plays a key role in the behavior of the tumor in this age that agree with Iraqi study (18). Also a group of researchers in both the United States and Australia that suggested the breast cancer incidence increases dramatically after the age of 40 (19).

Table (2): Mean of protein level according to age group

Age group(year)	Number of patients	Percentage according to age	Mean of protein level	P value
30-39	7	14%	251.4 ng/ml	0.60 NS
40-49	11	22%	241.1 ng/ml	
50-59	22	44%	242.4 ng/ml	
60-70	10	20%	183.0 ng/ml	
total	50	100%		

The results show no-significant associations between the high TGF-β1 protein levels and age, these results are corresponding with other demographic studies that suggested no significant different in protein level according to age (20).

Level of protein according to metastasis and non-metastasis patients

We analyzed the mean level of protein TGF-B1 in both metastasis and non metastasis patients. that was increase of the TGF-B1 level in both groups, the difference between them was statistically significant that agree with this study (21) that confirm the role of TGf-B1 in advanced breast cancer as a tumor promoter lead to Tumor growth, proliferation and migration that lead to metastasis pathway.

Table (3): Level of protein according to metastasis and non-metastasis.

Group	Metastasis	None metastasis
TGF-B1 mean protein level	Mean 5.581886782	Mean 2.0798837
	P-value: 0.00000142 *Significant*	

Conclusion

The observations from this study found that TGF-B1 protein level was increase in female with breast cancer compare to healthy women.

The mean age of the Iraqi breast cancer patients was above fifteen with more frequent between 50-59 years old. protein level of TGF-B1 The results also revealed that non-significant different in protein level of TGF-B1 between the age group but the cancer risk increase after 40 years old.

The protein Level of TGF-B1 has significant increase in Iraqi patients with breast cancer in metastasis breast cancer than none metastasis.

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