Lymphotoxin alpha gene expression in Iraqi breast cancer women

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ABSTRACT

Introduction and Aim: Women’s breast cancer is the number one cancer documented in Iraq, with cumulative evidence suggesting cytokines, including tumor necrosis factors probably contributing to the development of the disease. Thus, the aim of this study was to assess the contribution of Lymphotoxin alpha gene to breast cancer.

Materials and Methods: In this study, 100 women were enrolled; 50 breast cancer patients (25 women diagnosed with breast cancer but without treatment and 25 women who underwent surgery and different treatments); 25 women with benign tumours, and 25 apparently healthy women. Each participant’s blood sample (2 ml) was collected in a triazole-containing tube and stored at −20°C until use. For the detection of Lymphotoxin alpha gene expression, RNA was extracted from blood and subjected to qPCR.

Results: The study showed that 52% of breast cancer patients were over 50 years old among which 73% had attained menopause. The expression of the LTA gene was increased in breast cancer patients before treatment and in those with benign tumours (BT), which suggests that LTA might enhance the development of tumour cell breast cancer.

Conclusion: LTA expression may be associated to the development of breast cancer in women.

Keywords: LTA expression may be associated to the development of breast cancer in women.

INTRODUCTION

As the most prevalent malignancy in all Iraqi provinces, breast cancer (BC) is considered the primary cause of the high mortality rate among Iraqi women. According to the Iraqi Cancer Registry Annual Report, 35,864 new cancer cases were recorded in 2019, with an incidence rate of 91.66/100,000 individuals. Breast cancer accounts for 19.82% of all cancers (18.17/100,000 individuals), with females accounting for 34.08% (35.95/100,000 individuals) (1). Several malignancies, including breast cancer, have an inflammatory microenvironment that contributes to metastasis and is associated with escalating invasiveness and severe outcomes (2). Inflammatory mediators, such as tumour necrosis factor (TNF), are released throughout the inflammation process (4). Prolonged TNF production improves angiogenesis and affects tumour promotion through collaboration between stromal and tumour cells within the intercellular matrix (4). Previous reports indicated that the cancer patients developed high levels of TNF in their sera (5). Increase in TNF-α has been seen in many types of malignancies, particularly in breast cancer, with an increase being about 20-25% more than in healthy tissue. The high levels of TNF-α has been significantly correlated to tumour cell proliferation, high grade of malignancy, metastasis as well as the overall worse outcome of disease (6).

In contrast, T cells produce lymphotoxin alpha (LT-α), a pro-inflammatory cytokine that links lymphocytes and stromal cells before exerting its cytotoxic effect on cancerous cells. The synthesis of LTA is stimulated by interferons and IL-2. LTA is cytotoxic for a wide range of tumour cells both in vitro and in vivo (7). LT-α, also known as TNF-β, and TNF-α belong to the superfamily of tumor necrosis factor and share 30% similarity at the amino acid level. The TNF-α and TNF-β genes are located at proximity to each other within the MHC (HLA) locus on human chromosome 6 (8) and are closely linked. As with TNF-α, TNF-β gene has been implicated in tumour immunity, and it has been reported to inhibit carcinogenesis as well as in proliferation of cancer cells (7). Therefore, the purpose of this study was to examine the levels of TNF-β expression in breast cancer in a group of Iraqi women who were diagnosed with this cancer.

MATERIALS AND METHODS

The present study included 50 women with breast cancer (BC) (25 diagnosed but without treatment and 25 who were diagnosed and had undergone surgery and various treatments. In addition to 25 women with benign tumour (BT) and 25 apparently healthy women (control group -HC) were also included. The study participants were those that were admitted at Al-Amal National Hospital, Al Elwyra Hospital and Al Andalus
Hospital, in Baghdad from January to October 2021 and aged between 31-70 years. The present work was approved by the Ethics committee of the College of Science at University of Baghdad. PCR technique has widely succeeded in diagnosing different diseases related to genetics such as diagnosis of CML (9), adenocarcinoma (10), and beta-thalassemia (11).

Two millilitres of blood were collected from each participant in a triazole-containing tube and stored at –20°C until use. RNA was extracted from the blood samples using TRIzol™ Reagent (Thermo Scientific, USA). Concentration and purity of the extracted RNA was estimated using Nano-drop ND-1000 spectrophotometer (Nano-drop Technologies, Wilmington, USA). The real time-PCR steps were conducted using GoTag® 1-Step RT-qPCR System (Promega, USA) using the following primers: LTA-F: 5’-ACACCTTCA GCTGCCCA AGACTG-3’ and LTA-R: 5’-TCCGTGT TTGGTCTCCAGAGCA-3’. The β-globin housekeeping gene was also amplified using primers; β-Globin-F: 5’-ACACAACTGTGTTCACTA GC-3’ and β-Globin-R: 5’-CAACTTCATCCACGTT CACC-3’. A final volume of 10 μl reaction mixture contained 5 μl qPCR master mix, 0.25 μl RT mix, 0.25 μl MgCl2, 0.5 μl forward primer, 0.5 μl reverse primer, 2.5 μl nuclease-free water and 1 μl of RNA (25 ng/μl) was prepared. The thermal cycling conditions were as follows: one cycle RT, enzyme activation at 37°C for 15 minute, and initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C, annealing at 65°C, and extension at 75°C, each with a 2 second interval. The expression of the LTA gene was normalized to the housekeeping gene (β-globin). Each sample was analysed in duplicate. The \(2^{\Delta\Delta C_t}\) formula was applied to quantify gene expression (12). Data are presented as a number, percentage, and Mean ± SEM. All anthropometric and clinical parameters were compared among groups by one-way analysis of variance (ANOVA) test using SPSS version 23.0 (Chicago, IL, USA). The P values less than or equal to 0.05 were accepted to indicate a statistically significant difference.

**RESULTS**

Most breast cancer patients (52%) were over the age of 50, while 38% were between the ages of 30 and 50, and 12% were under the age of 30. In the benign group, 44% of women were between the ages of 30 and 40, 36% under 30, and 20% under 50. Among control women 44% were under 30-50 years of age, 40% under 50 years and 16% under <30 years of age. This difference was highly significant, P<0.001 as shown in Fig. 1. A significant number (73%) of breast cancer patients were menopausal, while 27% were premenopausal.

![Fig. 1: Distribution of study participants based on their age groups. BC: Breast cancer; BT: benign tumour; HC: healthy control](https://doi.org/10.51248/v42i6.2063)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>(\Delta C_t) (Mean ± SEM)</th>
<th>(2^{\Delta\Delta C_t}) (Mean ± SEM)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>20</td>
<td>21.64 ± 0.656</td>
<td>1.35 ± 0.459</td>
<td>S</td>
</tr>
<tr>
<td>BT</td>
<td>20</td>
<td>20.92 ± 0.554</td>
<td>4.82 ± 1.62</td>
<td>S</td>
</tr>
<tr>
<td>HC</td>
<td>20</td>
<td>21.64 ± 0.656</td>
<td>1.35 ± 0.459</td>
<td>S</td>
</tr>
<tr>
<td>BCBT</td>
<td>20</td>
<td>21.31 ± 0.803</td>
<td>3.56 ± 0.710</td>
<td>S</td>
</tr>
<tr>
<td>HC</td>
<td>20</td>
<td>21.64 ± 0.656</td>
<td>1.35 ± 0.459</td>
<td>NS</td>
</tr>
<tr>
<td>BCAT</td>
<td>20</td>
<td>22.90 ± 0.944</td>
<td>1.52 ± 0.638</td>
<td>NS</td>
</tr>
</tbody>
</table>

HC: healthy control; BT: benign tumour; BCBT: Breast cancer before treatment. BCAT: Breast cancer after treatment S: Significant; NS: Non-significant
LTA mRNA expression analysed as mean ± SEM for ∆Ct and fold change in BT, BCBT, BCAT patients and HC groups. The results showed that the ∆Ct mean of LTA gene decreased in BT and BCBT patients (20.92 ± 0.554 and 21.31 ± 0.803, respectively) and increased in BCAT (22.90 ± 0.944) compared to the corresponding ∆Ct means in controls (21.64 ± 0.656), with differences being not significant (p>0.05). However, there was a significant increase (p<0.01) in the LTA mRNA expression (2^∆∆Ct) in BT and BCBT patients (4.82 ± 1.62 and 3.56 ± 0.710, respectively) than in control (1.35 ± 0.459). No significant difference was observed for LTA mRNA expression between BCAT (1.52 ± 0.638) and the control group (1.35±0.459) (Table 1 and Fig. 2).

**DISCUSSION**

In general, the likelihood of having breast cancer increases with age. Obviously, this association is most common among menopausal women over the age of 50, with women in this age group accounting for around 80% of breast cancer cases (13). Upon menopause, estrogen production by the ovaries is reduced. The decrease in estrogen levels could lead to several endocrine disorders, including an increased risk of breast cancer in women (14, 15). Our study result is in line with other studies demonstrating the rarity of breast cancer in women under the age of 25; and to the fact that the incidence of breast cancer increases with age and remains constant among women aged 50 to 69 years (16). Our results for LTA (TNF-β) expression in breast cancer patients was compatible with previous reports that have shown that the TNF expression levels increase in many pre-neoplastic as well as malignant tissues (17). Furthermore, a significant association was reported between the polymorphism of TNF promoter and gene expression variation, many autoimmune disorders, microbial infections, and cancers (18). What’s more, the high level of TNF gene expression in pre-cancerous and tumour cells was also associated with various oncologic diseases progression like Barrett's adenocarcinoma, breast cancer, cervical carcinoma, chronic lymphocytic leukaemia, and prostate cancer (19). Besides lymphotoxins noted activities in the lymphoid organs development and upkeeping and its role in gastrointestinal immunity, LT-facilitated signalling through the NF-κB pathways were clarified to affect the growth and metastasis of cancer (20). Interestingly, a wide spectrum of genetic reports in humans demonstrated an association between low LTA gene expression and its genetic polymorphism (21). Notably in homozygous genotypes, a lower risk is associated with higher grades of bladder cancer, while the LTA polymorphism was associated with worse outcomes in B-cell lymphoma (22). Contrarily, the heterozygous genotypes in lung and endometrial tumours are associated with reduced incidence in comparison to those with homozygous genotypes (23). Such variability might be attributed to the pleiotropic activities of LT signalling in addition to the tissue-specific interactions with other intracellular signalling reactions (24). Of interest, LTA participates importantly in the regulation of innate immunity. Moreover, LTA contributes in preventing the growth of tumorous tissues and causing marked damage to malignant cell lines. Nevertheless, the uncontrolled LTA expression may lead to a continuous active signalling; which eventually results in unregulated cellular growth in addition to tumour development. The varied roles of LTA are mostly dependent on the body organ type, malignant cell type, sex, and duration of the immune response (25). These findings highlight the importance of breast cancer screening and early detection by demonstrating that breast cancer risk persists across the lifespan but is highest in women in their middle years.

**CONCLUSION**

In the present study, the LTA gene expression was seen to increase in women with breast cancer before treatment and in women with benign tumors which probably indicates a link between LTA expression and breast cancer. However, more studies are needed to elucidate the LTA contribution or other members of
the TNF superfamily in breast cancer development and prognosis.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES
