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Assessment of IGF-1 expression in the peripheral blood of women with recurrent breast cancer

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Abstract

Breast cancer is the most common malignancy affecting women worldwide. The insulin-like growth factor 1 (IGF-1) gene encodes a protein responsible for a wide variety of physiological processes, including differentiation and cell proliferation. Despite several studies on tumor tissues, no study has evaluated IGF-1 expression in the peripheral blood of women with recurrent breast cancer.

In this cross-sectional study, IGF-1 expression in the peripheral blood of 146 women with breast cancer treated approximately 5 years ago was quantified by quantitative reverse transcription polymerase chain. The women were divided into 2 groups: non-recurrence (n = 85) and recurrence (n = 61). Statistical analysis of the data was performed using ANOVA, Mann–Whitney, and Chi-squared tests ($P < .05$).

The results showed no significant difference in IGF-1 expression between the non-recurrence and recurrence groups ($P = .988$). In the subgroups of patients with lymph node involvement, no statistically significant difference was observed in IGF-1 expression between women with recurrence and those non-recurrence ($P = .113$). In patients without lymph node metastases, IGF-1 messenger ribonucleic acid (mRNA) expression levels were significantly higher in the non-recurrence group than in the recurrence group ($P = .019$). Furthermore, using the median IGF-1 mRNA expression as the cutoff point, it was obtained a statistically significant difference in tumor histological grade among women with recurrent breast cancer ($P = .042$).

These data showed significantly higher IGF-1 expression in women without lymph node metastases in the non-recurrence group compared with the recurrence group. In addition, a significant difference was observed in median IGF-1 mRNA expression in relation to tumor histological grade in women with recurrent breast cancer.

Abbreviations: IGF-1 = insulin-like growth factor 1, mRNA = messenger ribonucleic acid, RT-qPCR = quantitative reverse transcription polymerase chain reaction, TCGA = the Cancer Genome Atlas.

Keywords: breast cancer, gene expression, insulin-like growth factor 1, prognosis, recurrence

1. Introduction

With an estimated 2 million 89,000 new cases in 2018, breast cancer remains the most common malignancy among women worldwide, except in East Africa, where cervical cancer prevails.[1–3] In Brazil, a developing country, it is the most frequently diagnosed malignancy after non-melanoma skin cancer. There were an estimated 59,700 new breast cancer cases and 16,927 deaths from the disease in 2019.[4] Although physical

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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examination and mammography are essential to ensure early diagnosis of the disease and to reduce mortality, breast cancer is still often diagnosed at advanced stages in Brazil, resulting in high mortality rates despite current therapeutic strategies.[5]

Approximately 30% of the patients who are disease-free after initial treatment have disease recurrence during follow-up. The timing of breast cancer recurrence varies considerably under the influence of classic prognostic factors such as hormone receptor status, genetic changes, and lifestyle.[6] Therefore, breast cancer is a multifactorial disease, and one of the main risk factors is genetic alterations.[7–9] In this regard, one of the genes to draw attention due to its possible association with breast cancer tumorigenesis is the insulin-like growth factor 1 (IGF-1) gene. IGF-1 is located on the long arm of chromosome 12 and covers an area of over 80 kb of genomic DNA.[10] This gene encodes a protein of the same name that is produced in various organs of the body; however, the main source of circulating IGF-1 is the liver.[11] The IGF axis regulates a wide variety of physiological processes, including growth and development of normal human tissues by promoting cell proliferation and differentiation, and preventing apoptosis.[12,13]

IGF-1 is found in almost all human tissues, including normal and neoplastic breasts, and is fundamental for ductal morphogenesis and normal breast development.[14–16] The IGF-1 system is possibly involved in malignant transformation of normal breast cells, maintenance of malignant phenotype, increased metastatic potential, and resistance to apoptosis; these phenotypic characteristics lead to a worse prognosis for patients with breast cancer.[17]

The assessment of IGF-1 transcripts in cancer patients using qualitative methods, such as conventional reverse transcription polymerase chain reaction, has reported inconsistent and inconclusive results in relation to the levels of expression of IGF-1 mRNA in breast cancer.[18–20] Furthermore, the use of quantitative PCR, permits a highly sensitive quantification of IGF-1 expression, to investigate the correlation between this gene expression in the serum of breast cancer patients and susceptibility to tumor recurrence; however, there are controversies in the studies involving clinical features of breast cancer and the expression levels of IGF-1.[21–24]

Mu et al[25] showed a significant association between IGF-1 mRNA (messenger ribonucleic acid) expression levels and breast tumors smaller than 2 cm with low histological grade and favorable immunohistochemical profile. On the other hand, Chong et al[26] found no correlation between IGF-1 expression levels in breast tumors with clinicopathological and prognostic factors. Thus, controversies in IGF-1 expression in breast tumor studies mainly associated with RNA assessment have prompted us to investigate the expression of IGF-1 in the peripheral blood of women with recurrent breast cancer.

2. Patients and methods

2.1. Patients

This is a cross-sectional study, using the standard formula to calculate the sample size by prevalence. A total of 146 women aged between 34 to 80 years, diagnosed with breast cancer for approximately 5 years and enrolled for surgical treatment at the Mastology Clinic of the Perola Byington Hospital (Sao Paulo, Brazil), were recruited. Blood samples were collected during the follow-up between July and September 2018. The Internal Review Board of the Federal University of Piauí and Perola Byington Hospital approved the study under number CAAE: 43447015.8.0000 and all the patients signed an informed consent form prior to admission in the study, before surgery. All procedures performed in this study complied with current Brazilian laws and were in accordance with the ethical standards of the institutional and national research committees as well as the 1964 Helsinki declaration and its later amendments.

The patients were divided into 2 groups: breast cancer non-recurrence (n = 85) and with recurrence (n = 61). Women over 18 years of age, with and non-recurrence breast cancer (local, regional, and distant metastases) in the operable phase, diagnosed approximately 5 years ago and histologically proven cases (disease at diagnosis) were included in the study. Women with a history of another neoplasm, presence of a serious concomitant disease, and an initial diagnosis of metastatic breast cancer were excluded from the study.

2.2. Blood sampling

Peripheral blood was collected by a specialized technician using a disposable syringe and needle after medical consultation. Then, 0.3 mL sample of total peripheral blood from each patient was blocked with 0.9 mL TRIzol (Invitrogen) and stored at –80°C until RNA extraction.

2.3. Total RNA extraction and cDNA synthesis

RNA extraction was performed using TRIzol reagent (Invitrogen, Thermo Fisher Scientifc, Inc.) according to the manufacturer's instructions. RNA concentration, integrity and purity were analyzed using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.) and agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized from 2000 ng RNA using SuperScript III First-Strand Synthesis System (Invitrogen, Thermo Fisher Scientifc, Inc). The cDNA was kept at - 20°C and was diluted 10-fold prior to use in the RT-qPCR.

2.4. Quantitative reverse transcription polymerase chain reaction

IGF-1 mRNA expression was determined by RT-qPCR using the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, USA) and a 7500 Fast Real-Time PCR System (Applied Biosystems) equipped with SDS v1.4 software. The following primers were used for detection and quantification of IGF-1 mRNA: sense primer, 5'-GCTGGTGGATGCTCTT-CAGT -3'; antisense primer, 5'-ACTCATCCACGATG-CCTGTC -3'. Beta-actin was used as an endogenous normalization control. The following primers were used for beta-actin: sense primer, 5'-CACTGTGTTGGCGTACAGGT-3' and antisense primer, 5'-AAATCTGGCACCACACCTTC-3'. The melt curve was analyzed in order to check the reaction specificity and the relative IGF-1 expression was determined using the $2^{-\Delta CT}$ method.

2.5. IGF-1 expression in TCGA datasets and survival analysis

The Cancer Genome Atlas (TCGA) patient survival data for breast invasive carcinoma was retrieved from OncoLnc (Jordan Anaya, Charlottesville, USA; www.oncolnc.org),[27] a tool

containing cancer patient survival data in combination with RNA-seq data (https://www.cancer.gov/aboutnci/organization/ccg/research/structuralgenomics/tcga), in May 2020. The breast invasive carcinoma patients (n = 1006) were classified according to low or high IGF-1 expression and the Kaplan–Meier plots and *P*-values were generated.

2.6. Statistical analysis

To evaluate the associations between the relative expression of IGF-1 and the clinical and histopathological variables, the Mann–Whitney and unidirectional ANOVA tests were performed with multiple comparisons using the Bonferroni post-test method. To assess the associations between median expression of IGF-1 and clinical and histopathological variables, Chi-squared or Fisher exact tests were performed. Values of $P < .05$ were considered statistically significant. All statistical analyzes were performed using the GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA).

3. Results

3.1. Patient characteristics

The clinical-pathological characteristics of the groups with breast cancer non-recurrence, and with recurrence were considered homogeneous (Table 1).

Table 1

Clinical and histopathological characteristics of breast cancer women with and non-recurrence.

Variables Median age, 54 yr (range, 34–82 yr)	N (%)	Recurrence n (%)	Non-Recurrence n (%)	χ^2 (*P* value)
Age				
≤50 yr	60 (41.1)	25 (17.1)	35 (24.0)	.981
>51 yr	86 (58.9)	36 (24.7)	50 (34.2)	
Status				
Premenopausal	63 (43.2)	26 (17.8)	37 (25.3)	.913
Menopausal				
Postmenopausal	83 (56.8)	35 (24.0)	48 (32.9)	
Tumor Grade				
G1	14 (9.6)	4 (2.7)	10 (6.8)	.413
G2	107 (73.3)	48 (32.9)	59 (40.4)	
G3	25 (17.1)	9 (6.2)	16 (11.0)	
Tumor stage				
I	19 (13.0)	6 (4.1)	13 (8.9)	
II	74 (50.7)	31 (21.2)	43 (29.5)	.582
III	53 (36.3)	24 (16.4)	29 (19.9)	
Molecular subtype				
Luminal A	16 (11.0)	3 (2.1)	13 (8.9)	.286
Luminal B	66 (45.2)	29 (19.9)	37 (25.3)	
Her2 overexpression	23 (15.8)	10 (6.8)	13 (8.9)	
Triple Negative	31 (21.2)	13 (8.9)	18 (12.3)	
Hybrid Luminal	10 (6.8)	6 (4.1)	4 (2.7)	
Histological type				
Ductal	104 (71.2)	46 (31.5)	58 (39.7)	.422
Lobular	8 (5.5)	4 (2.7)	4 (2.7)	
Other	34 (23.3)	11 (7.5)	23 (15.8)	

G1 = grade 1, G2 = grade 2, G3 = grade 3, N = number.

3.2. Association of IGF-1 mRNA relative expression with clinical and histopathological characteristics

There was no statistically significant difference between the relative expression of IGF-1 mRNA in the recurrence group and that in the non-recurrence group ($P = .988$; Fig. 1A). After analysis of subgroups of patients with lymph node involvement, no statistically significant difference was observed in IGF-1 expression between the group with recurrence and the group non-recurrence ($P = .113$; Fig. 1B). However, after analysis of patients without lymph node involvement, IGF-1 mRNA expression was significantly higher in the non-recurrence group than in the

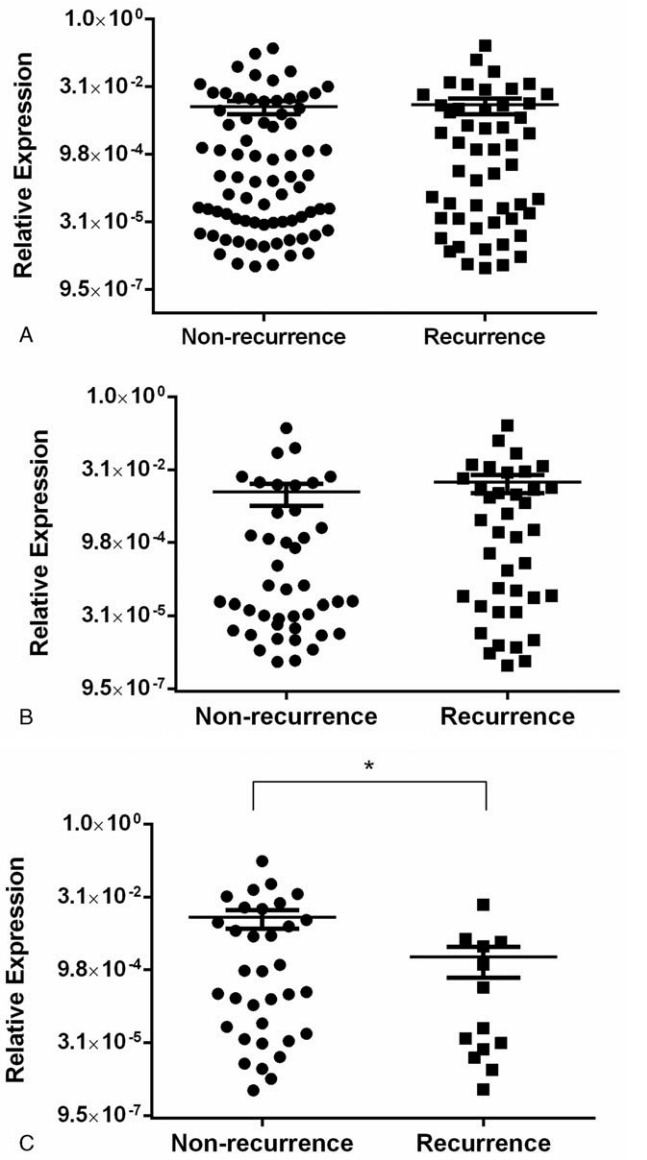

Figure 1. Relative expression of insulin-like growth factor 1 (IGF-1) mRNA. (A) Comparison of the relative expression of IGF-1 mRNA between the group with recurrence and the group with no recurrence ($P = .988$). (B) Relative expression of IGF-1 mRNA in patients with lymph node involvement, comparison between groups with recurrence and with no recurrence of breast cancer ($P = .113$). (C) Relative expression of IGF-1 mRNA in patients without lymph node involvement, comparison between groups with recurrence and with no recurrence of breast cancer (*$P = .019$).

Table 2

Association between peripheral blood insulin-like growth factor 1 messenger ribonucleic acid levels with clinical and pathologic characteristics of breast cancer patients with and non-recurrence.

Variables	Recurrence (n=61)			Non-Recurrence (n=85)		
	Low n (%)	High n (%)	*P* values	Low n (%)	High n (%)	*P* values
Age						
≤50 yr	12 (19.7)	13 (21.3)	.713	18 (21.2)	17 (20.0)	.958
>51 yr	19 (31.1)	17 (27.9)		26 (30.6)	24 (28.2)	
Status						
Premenopausal	12 (19.7)	14 (23.0)	.608	21 (24.7)	16 (18.8)	.512
Menopausal						
Postmenopausal	19 (31.1)	16 (26.2)		23 (27.1)	25 (29.4)	
Tumor Grade						
G1	0 (0.0)	4 (6.6)	.042*	6 (7.1)	4 (4.7)	.855
G2	28 (45.9)	20 (32.8)		30 (35.3)	29 (34.1)	
G3	3 (4.9)	6 (9.8)		8 (9.4)	8 (9.4)	
Tumor stage						
I	4 (6.6)	2 (3.3)	.447	5 (5.9)	8 (9.4)	.316
II	17 (27.9)	14 (23.7)		21 (24.7)	22 (25.9)	
III	10 (16.4)	14 (23.0)		18 (21.2)	11 (12.9)	
Molecular subtype						
Luminal A	1 (1.6)	2 (3.3)	.475	5 (5.9)	8 (9.4)	.735
Luminal B	16 (26.2)	19 (31.1)		23 (27.1)	18 (21.2)	
Her2 overexpression	5 (8.2)	5 (8.2)		7 (8.2)	6 (7.1)	
Triple Negative	9 (14.8)	4 (6.6)		9 (10.6)	9 (10.6)	
Histological type						
Ductal	23 (37.7)	23 (37.7)	.584	30 (35.3)	28 (32.9)	.507
Lobular	3 (4.9)	1 (1.6)		1 (1.2)	3 (3.5)	
Other	5 (8.2)	6 (9.8)		13 (15.3)	10 (11.8)	

G1 = grade 1, G2 = grade 2, G3 = grade 3, N = number.

* The values of $P < .05$ were interpreted as being statistically significant.

recurrence group ($P = .019$; Fig. 1C). Additionally, there was no association between the relative expression of IGF-1 mRNA and other variables (age, menopausal status, grade, tumor stage, hormone receptors, HER-2, and histological type; $P > .05$).

3.3. Association between peripheral blood IGF-1 mRNA levels with clinical and pathologic characteristics of breast cancer patients with and non-recurrence.

Using the median as the cutoff point, the relative expression of IGF-1 mRNA was classified as high and low, and then analyzed for association with clinical and histopathological features (Table 2). The group of women with breast cancer recurrence showed a significant difference in relation to tumor grade ($P = .042$), grade 1 is contributing to difference observed, since the frequency of grade 1 patients with high IGF-1 expression was much higher than the frequency expected by chance. There was no significant association between IGF-1 expression and the other variables studied ($P > .05$).

3.4. Expression of IGF-1 according to chemotherapy, radiotherapy and endocrinotherapy

The patients in the study received chemotherapy treatment (N = 144; 98,6%), radiotherapy (N = 115; 78.8%) and endocrinotherapy (N = 110; 75.3%). There was no statistically significant difference between the relative expression of IGF-1 mRNA in the recurrence group and that in the non-recurrence group, according to chemotherapy ($P = .996$), radiotherapy ($P = .166$), and endocrinotherapy ($P = .159$). Furthermore, no significant difference was observed in the median expression levels of IGF-1 mRNA between the recurrence group and the non-recurrence group, according to chemotherapy ($P = .794$; Fig. 2A), radiotherapy ($P = .103$; Fig. 2B) and endocrinotherapy ($P = .128$; Fig. 2C).

3.5. IGF-1 expression and breast cancer: an analysis using TCGA data

The IGF-1 expression and breast cancer was also evaluated usind TCGA data. The result suggested that IGF-1 expression assessed in breast invasive carcinoma tissue was not correlated with poor survival outcome ($P = .505$) as observed in the Kaplan plot generated by OncoLnc (Fig. 3).

4. Discussion

The detection of IGF-1 mRNA levels in breast cancer has been controversial. RT-qPCR is a sensitive and convincing method for mRNA detection in a variety of sites such as the lymph nodes, tissues, and blood.[28] However, according to studies in the literature so far, the evaluation of IGF-1 mRNA by RT-qPCR in the peripheral blood of women with and without relapsing breast cancer has not been reported; studies on only normal, peritumoral, and tumoral tissues are available.[29] Peripheral blood has been used as a clinical specimen to evaluate gene expression in breast cancer because in clinical practice, metastatic lesions are rarely biopsied due to their anatomical inaccessibility or the comorbidity associated with the procedure. Due to this, monitoring of blood biomarkers represents a new direction in

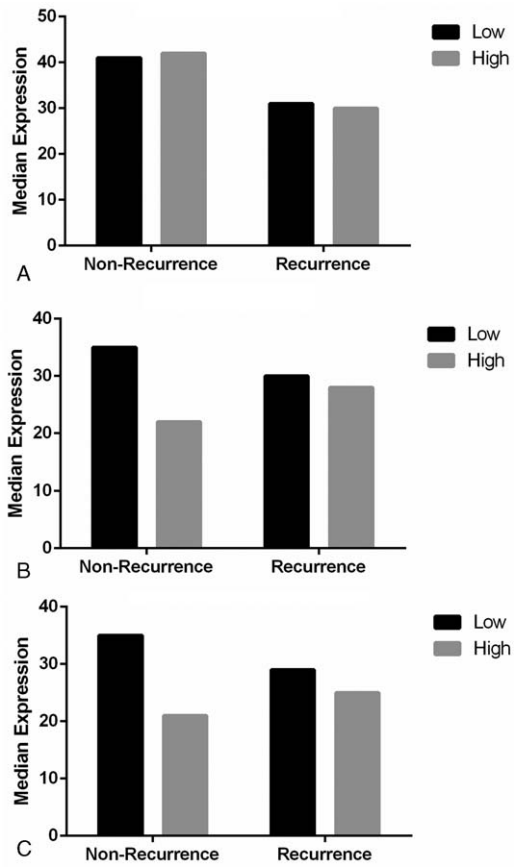

Figure 2. Median expression levels of insulin-like growth factor 1 (IGF-1). (A) Median expression levels of IGF-1 mRNA between the recurrence group and the non-recurrence group, according to chemotherapy ($P = .794$). (B) Median expression of IGF-1 transcripts between the recurrence group and the non-recurrence group, according to radiotherapy ($P = .103$), and (C) Median expression levels of IGF-1 mRNA between the recurrence group and the non-recurrence group, according to endocrinotherapy ($P = .128$).

developing a medical precision approach tailored to provide information on cancer diagnosis and prognosis.[30]

In the present study, the comparison of IGF-1 expression levels in the peripheral blood of women breast cancer non-recurrence and those with recurrence showed no statistically significant difference. However, after isolated analysis of patients without lymph node involvement, significantly higher IGF-1 mRNA expression was observed in the non-recurrence group than in the recurrence group. Regarding tumor characteristics, the group of women with breast cancer recurrence showed a statistically significant difference in the median expression of IGF-1 mRNA in relation to tumor grade. Conversely, there was no significant association between IGF-1 expression and the other variables studied, including the overall survival of breast cancer patients assessed by TCGA data, which suggests the relevance of evaluating the levels of IGF-1 in the peripheral blood in order to correlate its expression with clinical features.

Chong et al[26] studied IGF-1 mRNA levels in breast cancer patients with recurrence and reported lower IGF-1 mRNA levels compared to those in patients who remained free of recurrence. Mu et al[25] assessed IGF-1 mRNA levels in breast cancer tissue samples. Patients with high IGF-1 expression had a lower risk of disease recurrence compared to those with lower expression. However, in agreement with our results, Haffner et al[31] found no significant association with the risk of disease recurrence on evaluating IGF-1 mRNA levels in breast cancer tissue samples.

Chong et al[26] found no correlation between IGF-1 mRNA levels and lymph node status. However, some studies evaluating IGF-1 mRNA expression levels in mammary tumors and adjacent normal tissues have shown a significant association between IGF-1 expression and lymph node status [29,32] as well as the association of elevated IGF-1 mRNA levels with tumors without lymph node involvement[29]. In agreement with our results, Haffner et al[28] showed after isolated analysis of patients without lymph node involvement, that high IGF-1 mRNA expression was associated with a lower risk of recurrence. The hypothesis is that the IGF axis maintains some degree of tumor cell differentiation and less aggressiveness.[33]

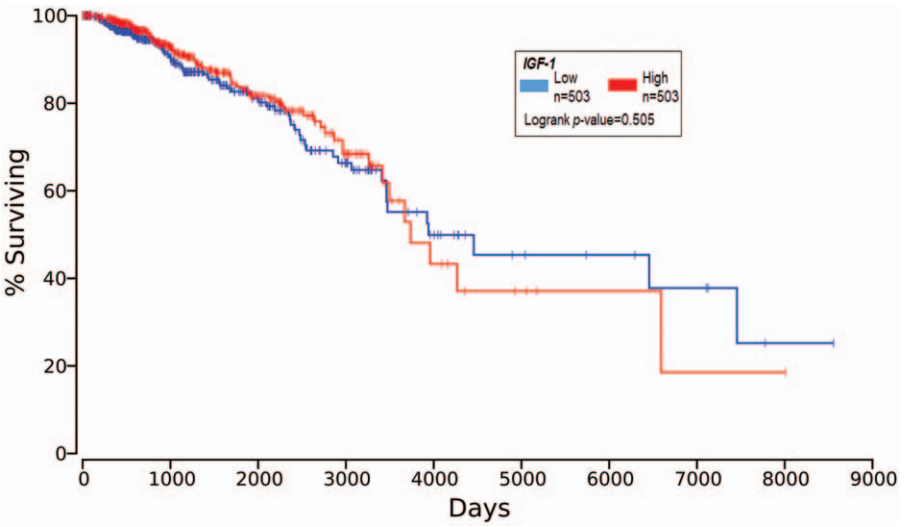

Figure 3. Kaplan–Meier plots showing the correlation between overall survival and high versus low insulin-like growth factor 1 expression in the tumor tissue of patients with invasive breast carcinoma (n = 1006; $P = .505$).

The histological status of axillary lymph nodes is an important prognostic factor. Patients with up to a single lymph node metastasis have worse outcomes than those with negative lymph nodes, and the decision on the most appropriate treatment is largely based on lymph node status. Risk stratification in patients without lymph node involvement is difficult. Therefore, there is a need for markers related to predicting outcomes in this group of patients.[31,34,35]

Sarakbi et al[32] evaluated IGF-1 mRNA expression in mammary tumors and found no significant association between IGF-1 expression and tumor grade, as reported by Chong et al.[26] However, in agreement with our results, Raval and Trivedi,[29] while evaluating IGF-1 levels in mammary tumors and adjacent normal tissues, showed a correlation between IGF-1 expression and tumor histological grade. In addition, Mu et al[33] showed that elevated IGF-1 mRNA levels are associated with low-grade tumors. Moreover, Mu et al[25] showed an association between IGF-1 gene expression and low histological grade tumors.

Contrastingly, regarding the clinical and tumor variables of the patients, Raval and Trivedi[29] showed that IGF-1 expression is inversely correlated with disease stage. They observed significant differences in IGF-1 expression in relation to age, menopausal status, tumor size, and disease stage. Furthermore, Mu et al[25] demonstrated that age is inversely associated with IGF-1 mRNA levels. Small tumors and disease stages have been associated with high IGF-1 mRNA expression, and hormone receptors have been positively correlated with IGF-1 expression. However, the results of some previous studies are in accordance with our findings, revealing no association between IGF-1 expression and variables such as age, tobacco use, menopausal status, tumor size, and estrogen receptor.[26,32]

Although in the study by Mu et al[33] all patients received surgery as a primary treatment and some also received adjuvant therapies, including 64.9% chemotherapy and 52.9% hormone therapy and breast radiotherapy for 58.3% of patients, the authors did not evaluate the expression of IGF-1 in relation to the treatment performed. However, similar to our findings Chong et al[26] found no statistically significant association between the expression of IGF-1 and the treatment performed for breast cancer.

In conclusion, the present study showed no significant difference in IGF-1 gene expression between the breast cancer recurrence and non-recurrence groups. After analysis of subgroups of patients with lymph node involvement, no statistically significant difference was observed in IGF-1 expression between women with recurrence and those non-recurrences. Contrastingly, in patients without lymph node involvement, IGF-1 mRNA expression was significantly higher in the non-recurrence group. In addition, a significant difference was observed in median IGF-1 mRNA expression in relation to tumor histological grade in women with recurrent breast cancer. Thus, our results reveal an association of IGF-1 gene expression in relation to lymph node status and tumor histological grade. However, to best assess tumor aggressiveness in women with and without recurrent breast cancer, further studies with larger sample sizes, and those including other characteristics such as body mass index, alcohol use, and physical inactivity, are needed.

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