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RESEARCH ARTICLE

Assessment of PI3KCA Immunohistochemical Expression and Tumor Infiltrating CD4+ and CD8+ Lymphocytes in Invasive Breast Carcinoma and Their Relation to Molecular Subtypes

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ABSTRACT

Background: Invasive breast carcinoma (IBC) exhibits multiple genetic and epigenetic alterations. Phosphatidylinositol-4, 5-biphospate- 3 -kinase, catalytic subunit alpha (PI3KCA) is frequently mutated in IBC and plays a key role in cell survival and growth. However, the role of PI3KCA protein expression in IBC and its subtypes is still controversial. Tumor infiltrating lymphocytes (TILs), including CD4+ and CD8+ T cells are important biomarkers in breast carcinoma and regulate tumor microenvironment. TILs expression levels, either high or low, are contradictory regarding IBC patients' survival and prediction of chemotherapy response. **Aims:** This retrospective study aimed to assess the immunohistochemical expression of PI3KCA, CD4, and CD8 in IBC patients. **Materials and Methods:** Paraffinized tissues were prepared from IBC patients (n=63) collected from Pathology Department, Faculty of Medicine, Tanta University. We assessed the expression of PI3KCA, CD4, and CD8 in relation to the available clinicopathological parameters and molecular subtypes of IBC. Moreover, we correlated immunohistochemical expression of PI3KCA to CD4/CD8 ratio in IBC cases. **Results:** Positive PI3KCA immunohistochemical expression, low positive CD4 and high positive CD8 T-lymphocytes were detected in 54%, 50.8%, and 49.2% of the studied cases, respectively, with statistically significant relations with higher tumor grade, advanced tumor stage, triple negative breast cancer (TNBC), lymphovascular and perineural invasion, and presence of major in-situ component. There was a significant strong negative correlation between PI3KCA expression and CD4/CD8 ratio. **Conclusions:** Positive expression of PI3KCA, low CD4+, high CD8+ T cells as well as low CD4/CD8 ratio in IBC correlate with unfavorable clinicopathological parameters. This study helps to evaluate the combined novel targeted therapy with immunomodulatory agents.

Keywords: Breast cancer, PI3KCA TILs, CD4/CD8 ratio

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INTRODUCTION

Breast carcinoma is the most common female cancer and a leading cause of cancer-related death among women. Breast carcinoma impacts 2.1 million women each year (Iensen et al, 2020). In Egypt, it represents about 33% of all cancer cases, with rising incidence among females (Abdelaziz et al., 2021). Carcinogenic mutations and categorizing breast carcinoma into molecular subtypes are interesting fields in cancer research. Moreover, studying the expression of novel markers in breast carcinoma has significant prognostic implications and aims to improve the therapeutic options (Pujania et al., 2020).

One of the most frequently mutated genes in invasive breast carcinoma (IBC) is phosphatidylinositol-4, 5-biphospate- 3 -kinase catalytic subunit alpha (PI3KCA) (Agahozo et al., 2019). PI3KCA encodes phosphoinositide 3 -kinase (PI3K) molecule which regulates extracellular growth signaling. PI3KCA controls many cellular functions such as cellular survival, proliferation, and differentiation. Also, PI3KCA can be considered as a transforming oncogene that plays a role in the carcinogenesis of breast,

colorectal, endometrial, and ovarian carcinomas (Elfgen et al., 2019).

PI3KCA mutations occur in about 16–45% of all IBC patients and are implicated in hormonal resistance. Thus, PI3KCA has been proposed as a promising therapeutic target (Agahozo et al., 2019). Tumor-infiltrating lymphocytes (TILs) are important cells in breast carcinoma and regulate the tumor microenvironment. In breast carcinoma. TILs are mainly T lymphocytes, especially CD8+ cytotoxic T-lymphocytes (CTLs), and CD4+ T helper cells. Specific CD8+ T-cell cytotoxic response depends on the activated CD4+ T helper cells (Wang et al., 2017a).

TILs have a controversial role in breast carcinoma. On one hand, TILs release various growth factors that induce both angiogenesis and lymphangiogenesis, permitting the dissemination of tumor cells. On the other hand, CD8+ T cells exert an antitumor effect through binding with the Fas ligand causing death of tumor cells. Also, TILs release many cytokines causing direct or indirect tumor cell lysis (Leong et al., 2022).

Exploring the relationship between the PI3KCA and TILs in invasive breast carcinoma is important to develop novel targeted therapy together with immunomodulatory agents (Sobral-Leite et al., 2019).

In this study, we aimed to evaluate the immunohistochemical expression of PI3KCA, CD4, and CD8 in invasive breast carcinoma and to study their expression in relation to the available clinicopathological parameters and molecular subtypes. Furthermore, PI3KCA immunohistochemical expression was correlated with CD4/CD8 ratio.

METHODS

Cases collection

This retrospective cross-sectional study was carried out on 63 specimens of invasive breast carcinomas, selected from the archives of Pathology Department, Faculty of Medicine, Tanta University. The research ethics committee, Faculty of Medicine, Tanta University approved this study (Approval code number: 34772). Inclusion criteria for our cases included female patients with available known hormonal profiles (estrogen receptor 'ER' and progesterone receptor 'PR'), Ki-67, and human epidermal receptor-2 (Her-2) status. The selected blocks were from the primary breast tumor. The patients underwent either conservative surgery with axillary lymph node dissection or modified radical mastectomy. Exclusion criteria included patients with recurrent tumor, or patients who received chemotherapy or any preceding treatment.

Collection of clinicopathological data

Patient's age, tumor histopathological type, molecular subtype, tumor grade, stage, as well as the presence of lymphovascular invasion, perineural invasion, and in situ component were obtained from archived pathological reports.

Histopathological examination

Formalin-fixed paraffin-embedded sections (4 µm thickness) were prepared for routine Hematoxylin & Eosin stain to confirm the histopathological diagnosis. Cases were classified according to the World Health Organization (WHO) classification of breast carcinoma 2019 [9]. Grading and staging were done according to WHO classification of breast carcinoma and the American Joint Committee on Cancer (AJCC) (Tan et al., 2020).

Immunohistochemical procedure

The immunostaining was performed for all cases using DAKO En Vision FLEX protocol in automated Link 48 DAKO AUTOSTAINER (DAKO/Agilent Corp.). For PI3KCA immune-staining; rabbit polyclonal antibody (ABclonal, Catalog. No. A0265) concentrated antibody (dilution 1:100) was used. TILs assessment was performed using Ready-to Use antibody CD4 and CD8 rabbit monoclonal antibody; Clone ARC0328 (ABclonal, Catalog. No. A19018) and Clone ARC55249 (ABclonal, Catalog. No. A22219), respectively.

Immunohistochemical evaluation of PI3KCA expression

PI3KCA was detected as cytoplasmic and/or nuclear staining. The staining results were evaluated semi-quantitatively by multiplying the intensity of staining and the percentage of stained cells (Wang et al., 2017b).

The percentage of stained cells was as follows: 0 (< 5% stained cells), 1 (5-25% stained cells), 2 (26-50% stained cells), 3 (51-75% stained cells), and 4 (76-100% stained cells). The staining intensity was then scored as: 0 (no staining), 1 (mild staining), 2 (moderate staining), and 3 (strong staining). The staining results were divided into 4 scores (ranging from 0-12): 0-3; negative, 4-6; weak positive (+1), 7-9; moderately positive (+2), and 10-12; strong positive (+3). The +1, +2, and +3 (4-12) scores were considered positive staining results.

Assessment of CD4 and CD8 TILs

Under microscope magnification of ×400, we evaluated the stromal TILs compartment and within the invasive tumor borders. TILs outside of the invasive tumor border and in areas with necrosis or crushing artifacts, TILs around normal breast lobules or ductal carcinoma in situ, were excluded. Lymphoid follicles and peritumoral TILs were also excluded (Tan et al., 2020). Regarding TILs count, we evaluated three fields.

The number of immunoreactive cells in these areas was counted to calculate the mean for each case. Counting of TILs was performed using Fiji image analysis software (http://fiji.sc), "cell counter" plugin (Figure 1). The median TILs value was used as a cut-off point to classify the cases into low and high TILs groups. For each case, CD4/CD8 ratio was calculated. The cutoff point was calculated as the median value of studied cases; to group the cases into low vs. high CD4/CD8 ratio.

Statistical analysis

Statistical analysis of the data was done using statistical package for the social sciences software version 23.0 (TBM corp., Armonk, New York., USA). Quantitative data were described as range, mean, median, and standard deviation (SD). Chi square ($\chi 2$), Fisher Exact (FE), and Monte Carlo (MC) tests were used to assess the relation between the qualitative variables. Pearson correlation coefficient was used to evaluate the correlation between PI3KCA expression and CD4/CD8 ratio. The significance of the results was judged at 5% level ($P<0.05$).

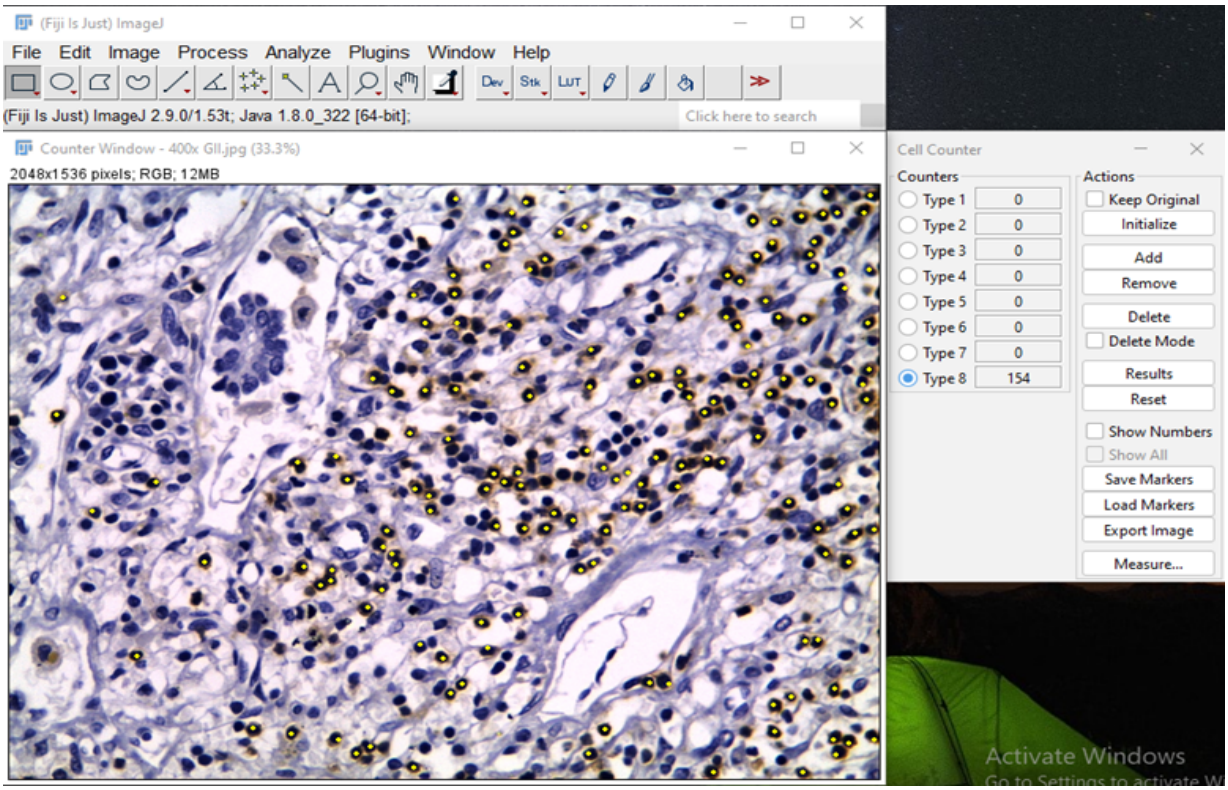

Figure 1. Fiji Image J software (a window of cell counter plugin): CD4+ T cells count in grade II invasive ductal carcinoma of no special type (IDC-NST) [154 cell/HPF x400] HPF, high-power field

RESULTS

The present study was performed on 63 cases of invasive breast carcinoma. All cases were female. The age range of the studied cases was from 18 and 72 years with a mean of 50.2 ± 10.8 and median of 52 years. Table 1 summarizes the clinicopathological data of the studied cases.

PI3KCA immunohistochemical expression in the studied cases

Regarding PI3KCA expression, 34/63 (54%) showed positive PI3KCA immunohistochemical expression (Figure 2).

Table 1. Clinicopathological data of studied invasive breast carcinoma cases

Clinicopathological parameters	Number [%]
Histopathological types	
IDC-NST	35 [55.6]
ILC	11 [17.5]
Metaplastic carcinoma	6 [9.5]
Mucinous carcinoma	5 [7.9]
Invasive papillary carcinoma	4 [6.3]
Carcinoma with apocrine differentiation	2 [3.2]
Histopathological grades	
Grade II	28 [44.4]
Grade III	35 [55.6]
Tumor size (T)	
T1: < 2cm	11 [17.5]
T2: 2-5cm	36 [57.1]
T3: > 5cm	16 [25.4]
Axillary lymph node status (N)	
N0	21 [33.3]
N1	19 [30.2]
N2	17 [27]
N3	6 [9.5]
Tumor stage	
Stage I	8 [12.7]
Stage II	22 [34.9]
Stage III	33 [52.4]
Molecular subtypes	
Luminal A	18 [28.6]
Luminal B	12 [19]
TNBC	33 [52.4]
Lymphovascular invasion	
Positive	35 [55.6]
Negative	28 [44.4]
Perineural invasion	
Positive	27 [42.9]
Negative	36 [57.1]
Carcinoma in situ component	
Major component	28 [44.4]
Minor component	35 [55.6]

IDC-NST, Invasive Ductal Carcinoma of No Special Type, ILC, Invasive Lobular Carcinoma, TNBC, triple negative breast cancer

Positive PI3KCA expression showed statistically significant relation with higher tumor grade (P<0.0001), advanced tumor stage (P=0.0028), triple negative breast cancer (TNBC) (P=0.00065), the presence of lymphovascular invasion (P<0.0001) and perineural invasion (P=0.0019), as well as the presence of major in-situ component (P<0.0001). PI3KCA immunohistochemical results are summarized in Table 2.

CD4 and CD8 TILs immunohistochemical expression in the studied cases

The cut off value of CD4 expression was 70 CD4+ T cells/HPF. High CD4 expression showed statistically significant relation with lower tumor grade (P<0.0001), early tumor stage (P=0.0002), luminal subtype breast cancer (P=0.0067), absence of lymphovascular invasion (P<0.0001) and perineural invasion (P<0.0001), as well as the presence of minor in-situ component (P<0.0001) (Figure 3). Regarding CD8 expression, the cut off value of CD8 expression was 60 CD8+ T cells/HPF. High CD8 expression showed statistically significant relation with higher tumor grade (P < 0.0001), advanced tumor stage (P=0.0074), the presence of lymphovascular invasion (P<0.0001), as well as the presence of major in-situ component (P=0.04) (Figure 4). CD4 and CD8 immunohistochemical results are summarized in Table 3.

CD4/CD8 ratio of the studied cases

The cut-off value of CD4/CD8 ratio was 1.3 and accordingly, the ratio is divided into high CD4/CD8 ratio > 1.3 and low CD4/CD8 ratio ≤ 1.3. High CD4/CD8 ratio was found in 31/63 cases (49.2%). High CD4/CD8 ratio showed a statistically significant relation with lower tumor grade (P<0.0001), early tumor stage (P<0.0001), luminal subtype breast cancer (P<0.0001), absence of lymphovascular invasion (P<0.0001) and perineural invasion (P=0.0107), and presence of minor in-situ component (P=0.0009). CD4/CD8 ratio results are summarized in Table 4.

Correlation between PI3KCA and CD4/CD8 ratio

The correlation between PI3KCA expression and CD4/CD8 ratio of the studied cases was assessed using Pearson correlation coefficient.

Table 2. PI3KCA immunohistochemical expression in the studied cases in relation to clinicopathological parameters

Clinicopathological parameters	PI3KCA		P-value
	Negative expression (%)	Positive expression (%)	
Histopathological type			
IDC-NST	18 (51.4)	17 (48.6)	0.31^{MC}
ILC	5 (45.5)	6 (54.5)	
Metaplastic carcinoma	0 (0.0)	6 (100)	
Mucinous carcinoma	3 (60)	2 (40)	
Invasive papillary carcinoma	2 (50)	2 (50)	
Carcinoma with apocrine differentiation	1 (50)	1 (50)	
Histopathological grade			
Grade II	22 (78.6)	6 (21.4)	< 0.0001* X^2
Grade III	7 (20)	28 (80)	
Tumor stage			
I	7 (87.5)	1 (12.5)	0.0028*^{MC}
II	13 (59.1)	9 (40.9)	
III	9 (27.3)	24 (72.7)	
Molecular subtype			
Luminal A	11 (61.1)	7 (38.9)	0.00065*^{MC}
Luminal B	10 (83.3)	2 (16.7)	
TNBC	8 (24.4)	25 (75.8)	
Lymphovascular invasion			
Positive	6 (17.1)	29 (82.9)	< 0.0001* X^2
Negative	23 (82.1)	5 (17.9)	
Perineural invasion			
Positive	6 (22.2)	21 (77.8)	0.0019* X^2
Negative	23 (63.9)	13 (36.1)	
Carcinoma in-situ component			
Major in-situ component	3 (10.7)	25 (89.3)	< 0.0001*^{FE}
Minor in-situ component	26 (74.3)	9 (25.7)	

PI3KCA: Phosphatidylinositol-4, 5-biphospate- 3 -kinase, catalytic subunit alpha- IDC-NST, Invasive Ductal Carcinoma of No Special Type, ILC, Invasive Lobular Carcinoma, TNBC, triple negative breast cancer, X^2, Chi-square test, FE, Fisher's exact test, MC, Monte Carlo test, * Statistically significant at P-value < 0.05

Table 3. CD4 and CD8 immunohistochemical expression in the studied cases and their relation to clinicopathological parameters

Clinicopathological parameters	CD4 expression (%)		P-value	CD8 expression (%)		P-value
	High	Low		High	Low	
Histopathological type						
IDC-NST	20 (57.1)	15 (42.9)	0.49^{MC}	15 (42.9)	20 (57.1)	0.6^{MC}
ILC	4 (36.4)	7 (63.6)		6 (54.5)	5 (45.5)	
Metaplastic carcinoma	1 (16.7)	5 (83.3)		5 (83.3)	1 (16.7)	
Mucinous carcinoma	3 (60)	2 (40)		2 (40)	3 (60)	
Invasive papillary carcinoma	2 (50)	2 (50)		2 (50)	2 (50)	
Carcinoma with apocrine differentiation	1 (50)	1 (50)		1 (50)	1 (50)	
Histopathological grade						
Grade II	22 (78.6)	6 (21.4)	< 0.0001*	5 (17.9)	23 (82.1)	< 0.0001* X^2
Grade III	9 (25.7)	26 (74.3)		26 (74.3)	9 (25.7)	
Staging						
I	8 (100)	0 (0.0)	0.0002*^{MC}	1 (12.5)	7 (87.5)	0.0074*^{MC}
II	14 (63.6)	8 (36.4)		8 (36.4)	14 (63.6)	
III	9 (27.3)	24 (72.7)		22 (66.7)	11 (33.3)	
Molecular subtypes						
Luminal A	13 (72.2)	5 (27.8)	0.0067*^{MC}	5 (27.8)	13 (72.2)	0.94^{MC}
Luminal B	8 (66.7)	4 (33.3)		3 (25)	9 (75)	
TNBC	10 (30.3)	23 (69.7)		10 (69.7)	23 (30.3)	
Lymphovascular invasion						
Positive	7 (20)	28 (80)	<0.0001*^{FE}	27 (77.1)	8 (22.9)	< 0.0001*^{FE}
Negative	24 (85.7)	4 (14.3)		4 (14.3)	24 (85.7)	
Perineural invasion						
Positive	5 (18.5)	22 (81.5)	< 0.0001*	17 (63)	10 (37)	0.077 X^2
Negative	26 (72.2)	10 (27.8)		14 (38.9)	22 (61.1)	
Carcinoma in-situ component						
Major in-situ component	4 (14.3)	24 (85.7)	<0.0001*^{FE}	18 (64.3)	10 (35.7)	0.04* X^2
Minor in-situ component	27 (77.1)	8 (22.9)		13 (37.1)	22 (62.9)	

IDC-NST, Invasive Ductal Carcinoma of No Special Type, ILC, Invasive Lobular Carcinoma, TNBC, triple negative breast cancer X^2, Chi-square test, FE, Fisher's exact test, MC, Monte Carlo test, * Statistically significant at P-value < 0.05

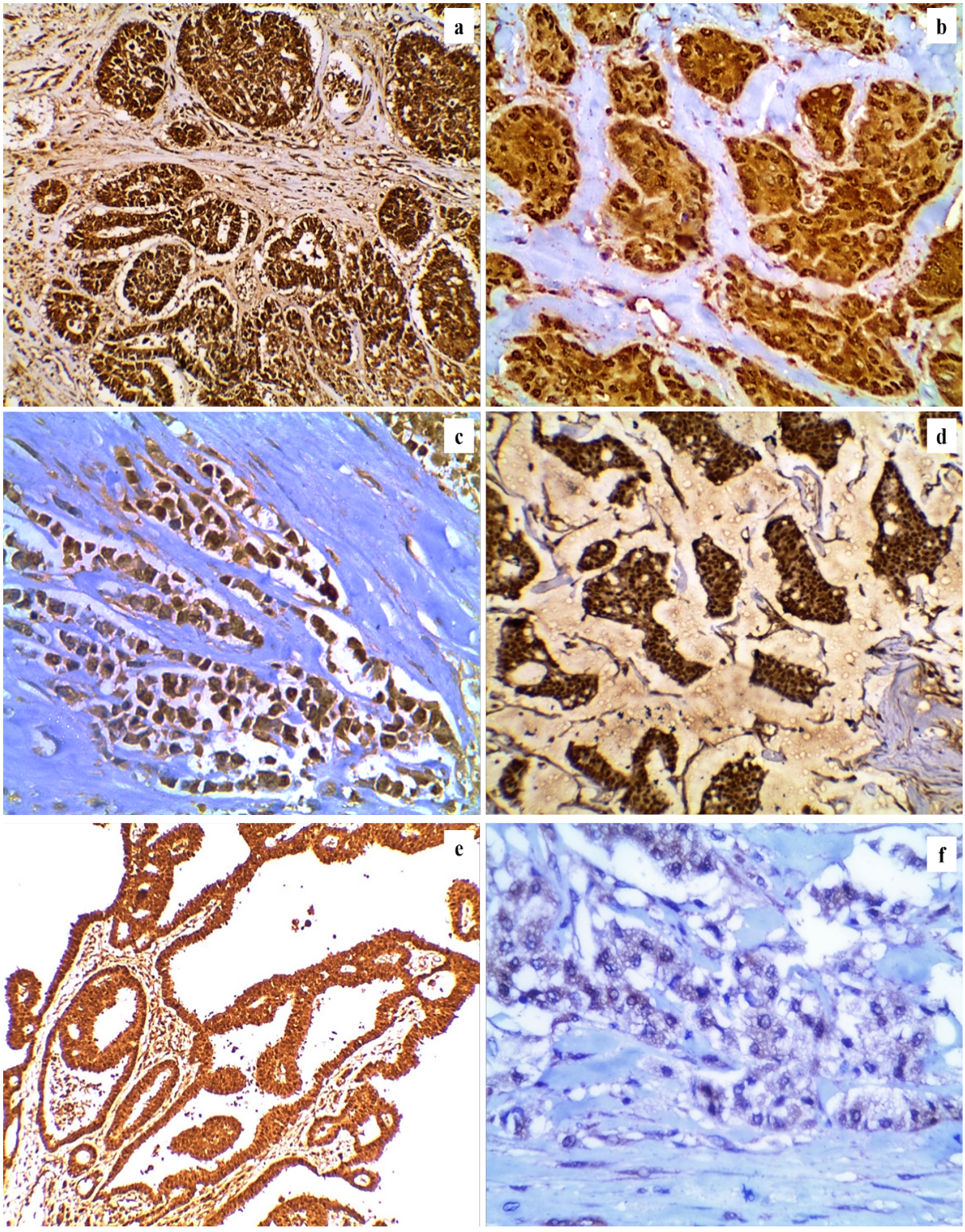

Figure 2. Phosphatidylinositol-4, 5-biphospate- 3 -kinase, catalytic subunit alpha (PI3KCA) immunohistochemical expression in breast carcinoma specimens. Positive PI3KCA expression in invasive ductal carcinoma of no special type, grade II (x200) (a), Positive PI3KCA expression in invasive ductal carcinoma of no special type, grade III (x400). (b), Positive PI3KCA expression in invasive lobular carcinoma (x400) (c), Positive PI3KCA expression in mucinous carcinoma, grade II (x200) (d), Positive PI3KCA expression in invasive papillary carcinoma (x200) (e), Negative PI3KCA expression in invasive ductal carcinoma-of no special type, grade II (x400) (f)

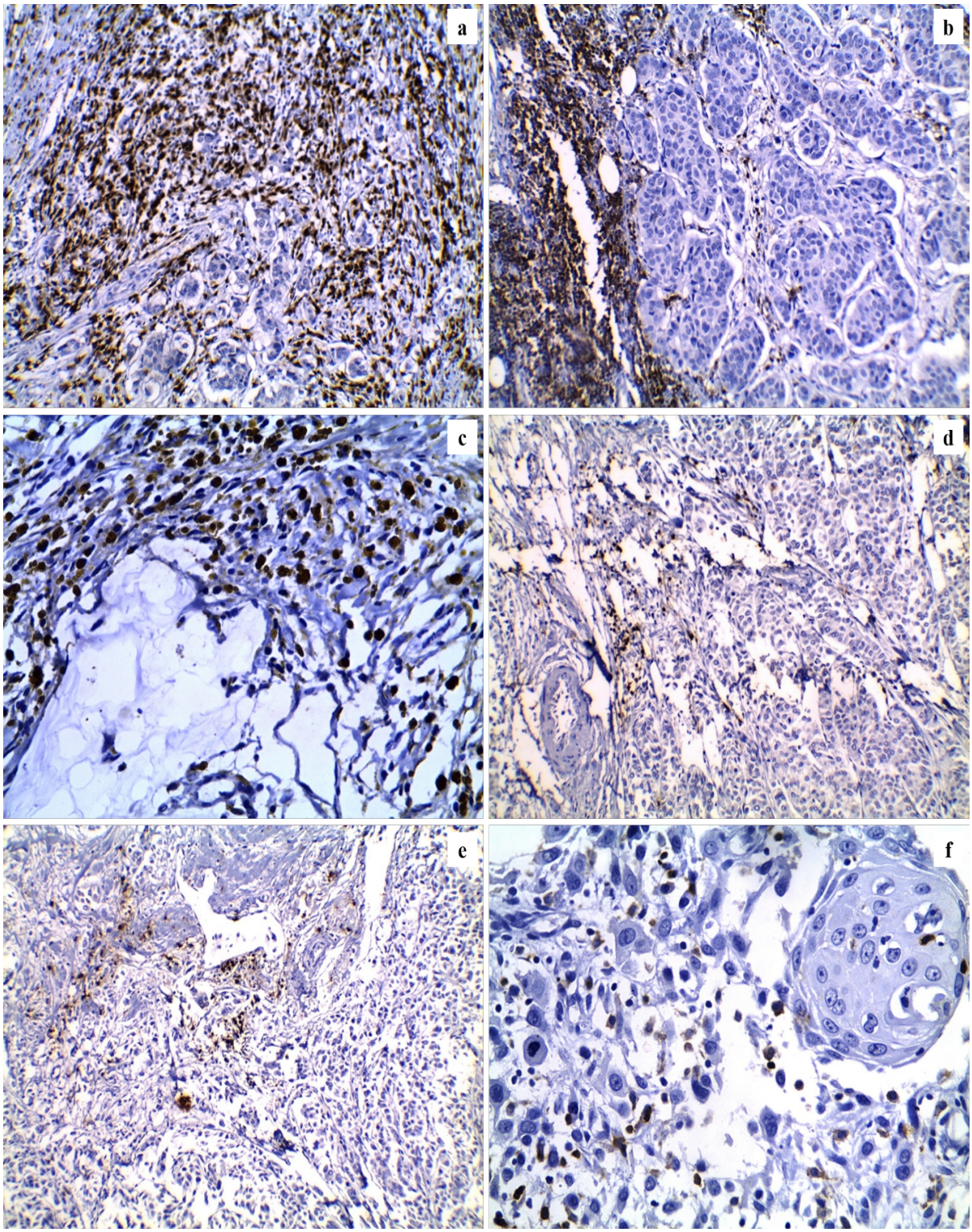

Figure 3. CD4 immunohistochemical expression in breast carcinoma specimens. High positive CD4 in invasive ductal carcinoma-of no special type, grade II (x200) (a), High positive CD4 in invasive ductal carcinoma-of no special type, grade III (x200) (b), High positive CD4 in mucinous carcinoma (x400) (c), Low positive CD4 in invasive ductal carcinoma-of no special type, grade III (x200) (d), Low positive CD4 in pleomorphic lobular carcinoma (x200) (e), Low positive CD4 in metaplastic carcinoma (x400) (f)

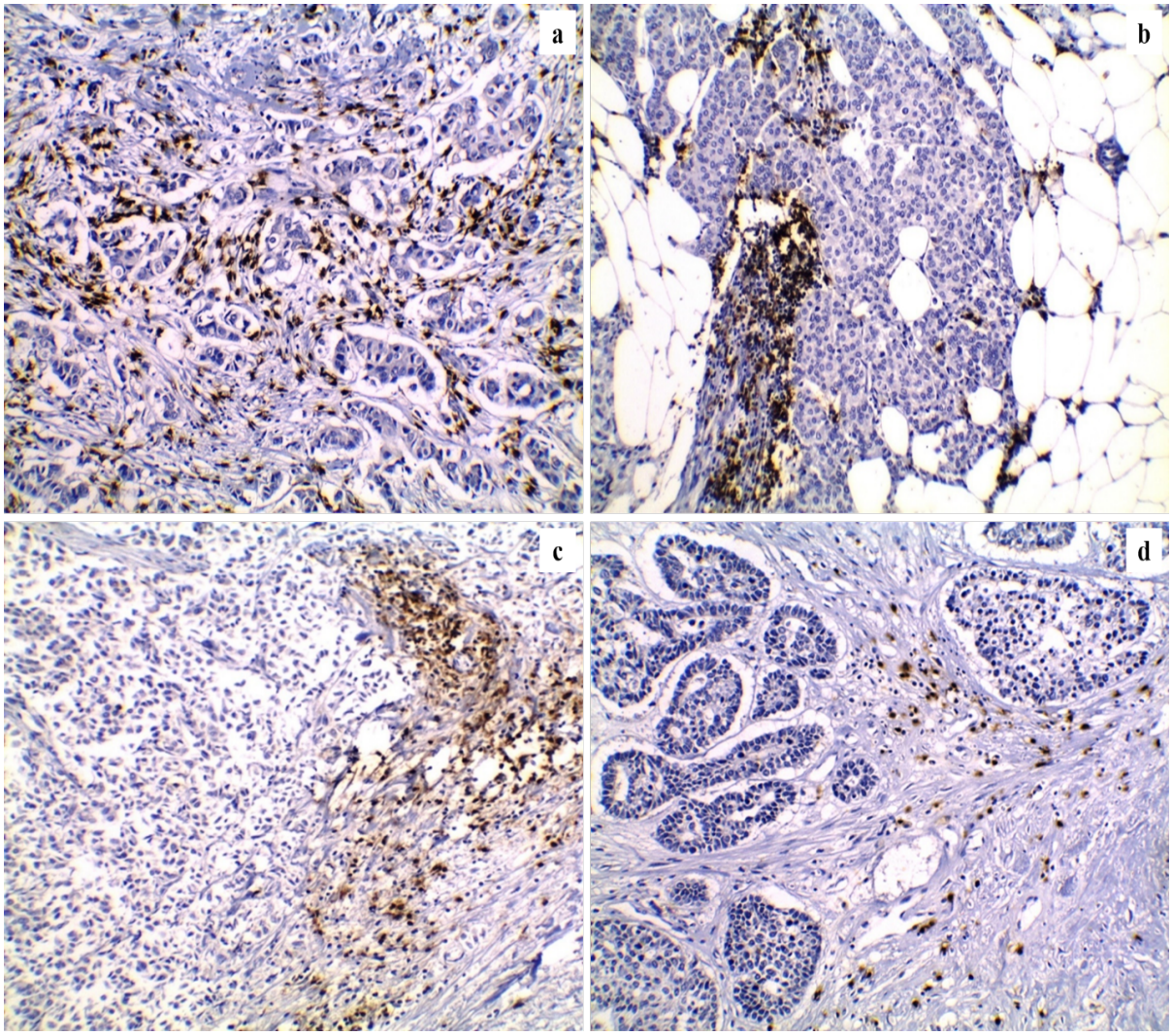

Figure 4. CD8 immunohistochemical expression in breast carcinoma specimens. High positive CD8 in invasive ductal carcinoma-of no special type, grade II (x200) (a), High positive CD8 in invasive ductal carcinoma-of no special type, grade III (x200) (b), High positive CD8 in invasive lobular carcinoma (x200) (c), Low positive CD8 in invasive ductal carcinoma-of no special type, grade II (x200) (d)

There was a strong negative correlation between PI3KCA expression and CD4/CD8 ration (P=0.01, r=-0.894) (Table 5 and Figure 5).

DISCUSSION

Invasive breast carcinoma (IBC) exhibits different histological features, molecular signatures, as well as different biological behaviors and variable prognoses. Exploring different prognostic and predictive factors in IBC patients is important to provide appropriate clinical management (Shaoxian et al., 2017).

In the present study, we evaluated the immunohistochemical expression of PI3KCA, CD4, and CD8 in invasive breast carcinoma in relation to the available clinicopathological parameters and molecular subtypes. Moreover, we studied PI3KCA immunohistochemical expression in relation to CD4 and CD8 expression in invasive breast carcinoma cases. In the current study, positive PI3KCA expression was detected in 54% of cases. There was insignificant relation between PI3KCA expression and the histopathological types which was in concordance with Elfgen et al.,2019 and Mosele et al., 2020 who stated that there was insignificant relation between PI3KCA expression and the histopathological types of IBC.

On the other hand, Shimoi et al., 2018 stated that there was a significant relation between PI3KCA expression and the histopathological types.

Table 4. CD4/CD8 ratio of the studied cases and its relation to clinicopathological parameters

Clinicopathological parameters	CD4/CD8 ratio (%)		P-value
	High	Low	
Histopathological type			
IDC-NST	19 (54.3)	16 (45.7)	0.67[MC]
ILC	5 (45.5)	6 (54.5)	
Metaplastic carcinoma	1 (16.7)	5 (83.3)	
Mucinous carcinoma	3 (60)	2 (40)	
Invasive papillary carcinoma	2 (50)	2 (50)	
Carcinoma with apocrine differentiation	1 (50)	1 (50)	
Histopathological grade			
Grade II	26 (92.9)	2 (7.1)	< 0.0001*[FE]
Grade III	5 (14.3)	30 (85.7)	
Tumor stage			
I	8 (100)	0 (0.0)	< 0.0001*[MC]
II	17 (77.3)	5 (22.7)	
III	6 (18.2)	27 (81.8)	
Molecular subtypes			
Luminal A	16 (88.9)	2 (11.1)	< 0.0001*[MC]
Luminal B	9 (75)	3 (25)	
TNBC	6 (18.2)	27 (81.8)	
Lymphovascular invasion			
Positive	6 (17.1)	29 (82.9)	< 0.0001*[FE]
Negative	25 (89.3)	3 (10.7)	
Perineural invasion			
Positive	8 (29.6)	19 (70.4)	0.0107*[X^2]
Negative	23 (63.9)	13 (36.1)	
Carcinoma in-situ component			
Major in-situ component	7 (25)	21 (75)	0.0009*[X^2]
Minor in-situ component	24 (68.6)	11 (31.4)	

IDC-NST, Invasive Ductal Carcinoma of No Special Type, ILC, Invasive Lobular Carcinoma, TNBC, triple negative breast cancer, X^2, Chi-square test, FE, Fisher's exact test, MC, Monte Carlo test,
* Statistically significant at P-value < 0.05

Table 5. Correlation between PI3KCA expression and CD4/CD8 ratio

PI3KCA	Total	High CD4/CD8 ratio (%)	Low CD4/CD8 ratio (%)
Negative	29	24 (82.8)	5 (17.2)
Positive	34	7 (20.6)	27 (79.4)
Total	63	31 (49.2)	32 (50.8)
P-value	0.01*		

PI3KCA: Phosphatidylinositol-4, 5-biphospate- 3 -kinase, catalytic subunit alpha
* Statistically significant at P-value < 0.05

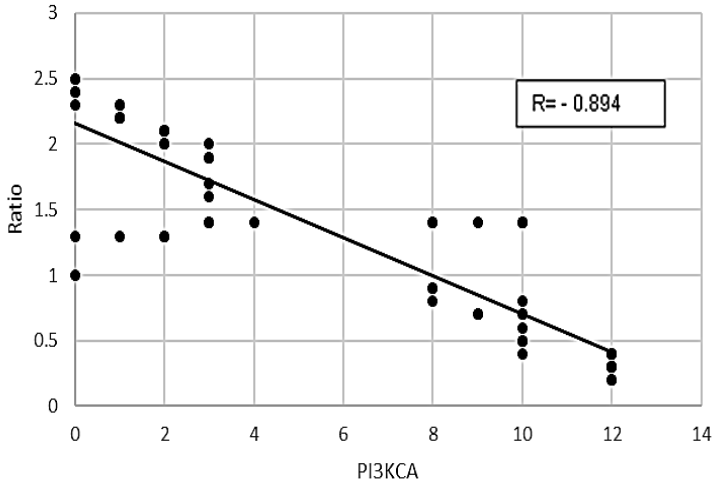

Figure 5. Correlation between PI3KCA and CD4/CD8 ratio in the studied cases

There was a statistically significant relation between expression and the histological grade of the studied cases, where PI3KCA expression increased with grade III.

The same results were obtained by Elfgen et al., 2019 who found that 60.8% of grade III showed positive PI3KCA expression. Wang et al., 2017b found that 74.42% of grade III cases expressed PI3KCA. On the contrary, Reinhardt et al., 2022 reported that PI3KCA expression decreased with advancing histological grade (15.8% of grade III cases). Their explanation was that PI3KCA-mutations were considered one of the early events in breast carcinogenesis as they occurred even in small tumors and that the fast-growing undifferentiated tumors were independent of activating PI3KCA-mutations.

This study reported a significant relation between PI3KCA expression and tumor stage. In agreement with our results, Wang et al., 2017b stated that PI3KCA was highly expressed in breast cancer associated with axillary lymph nodal metastasis and was also strongly associated with larger tumor size. The relation between the molecular subtypes and PI3KCA in our study was statistically significant. The current study revealed positive PI3KCA expression in 75% of triple negative cases followed by luminal subtypes. Similarly, Cho et al., 2022 revealed high prevalence of PI3KCA mutation across triple-negative breast carcinoma (57.6%). Also, Prvanović et al., 2021 stated that PI3KCA was highly expressed in TNBC (97% of cases). Unlike our results, Reinhardt et al., 2022 stated that the PI3KCA gene mutations were detected in tumors with luminal molecular subtypes, while tumors with TNBC showed the lowest rate of PI3KCA-mutations (11.4%). They postulated that PI3KCA may lead to estradiol-independent ER transcriptional activity. Therefore, PI3KCA mutations may induce the growth of ER-positive tumors.

Our study revealed a significant relationship between PI3KCA and lymphovascular invasion. This was in concordance with Prvanović et al., 2021 and Hosoya et al., 2023 who showed that high PI3KCA expression levels were associated with lymphatic vessel metastasis. PI3KCA may promote the over-expression of vascular endothelial growth factor-3 (VEGFR-3) in breast cancer cells and may induce lymphangiogenesis and lymphovascular invasion.

In this study, about 89.3% of cases with major carcinoma in situ expressed PI3KCA. In the same line with our study, Zardavas et al., 2018 observed that PI3KCA mutations were expressed in ductal carcinoma in situ and therefore, PI3KCA mutation may be an early event in breast cancer development preceding invasion. Tumor-infiltrating lymphocytes (TILs) are important prognostic markers in various tumors. Therefore, the relation between immune infiltration and invasive breast carcinoma has received great attention (Tan et al., 2020).

Regarding the relation between CD4 expression and clinicopathological parameters of the studied cases, we found that high CD4 positive T-cells was significantly observed in cases with lower grade and stage, luminal subtypes, absence of lymphovascular and perineural invasion, and minor carcinoma in-situ component.

Similar to our results, Matkowski et al., 2009 stated that CD4+ T cell number was higher in N0 than in N+ invasive breast carcinomas. Sheu et al., 2008 also stated that lymphovascular invasion and the advanced stage was associated with a decreased number of CD4+ T cells. Seo et al., 2013 showed that a high count of CD4+ T cells was associated with less advanced clinical stage. Wang et al., 2017a also stated that increased CD4+ T cell count was associated with smaller tumor size and lower tumor stage favoring better survival outcomes. Li et al., 2023 reported that high CD4+ T cells infiltration is associated with good prognostic effects in luminal molecular subtypes.

Supporting our results, Wang et al., 2017a stated that CD4+ T cells release certain cytokines that could increase T cell infiltrate enhancing the immune response. Also, CD4+ T-cells could induce, maintain, and memorize CD8+ CTLs to enhance their cytotoxic activity. Moreover, Rathore et al., 2014 reported that CD4+ T cells could acquire cytotoxic properties to mediate tumor cell killing, even in the absence of CD8+ T cells.

On the other hand, Matkowski et al., 2009 showed that no associations between CD4+ T cells expression and tumor size, histological grade, ER, PR and HER-2 expressions were observed. Seo et al., 2013 stated that high CD4+ T cell count was strongly associated with high histological grade and triple-negative subtype.

Rathore et al., 2014 also found that higher numbers of CD4+ T cells were significantly associated with high grade, advanced stage, and presence of lymphovascular invasion. They stated that during cancer progression, the CD4+ T cells may change from effector to suppressor phenotype. This change may reduce the antigen expression level, leading to tumor tolerance and persistence. Unlike CD4, high CD8+ T cells in the current study were associated with advanced grade and stage, lymphovascular invasion, and major carcinoma in situ components. Meanwhile, CD8+ T cells showed insignificant relation with the histopathological types, the molecular subtypes of breast cancer, and perineural invasion.

Similar to our result, Sheu et al., 2008 reported that CD8+ T cells in lymph node-positive cases, expression and tumor size, histological grade, ER, PR and HER-2 expressions. Seo et al., 2013 said that the CD8+ T cells showed no relation with clinical stage. Jafarian et al., 2018 reported that CD8+ T cells were not significantly related to various tumor grades, but high CD8+ T cells were associated with significantly reduced lymph nodal involvement.

Huang et al., 2015 stated that high CD8+ T cells were associated with early tumor stage, smaller tumor size, and negative lymph node status. The clear difference between his study and ours is that he chose a different cut-off value. His CD8+ T cells cut-off value was 13 T-cells/HPF while our CD8+ T cut-off value was 60 T-cells/HPF.

In this study, high CD4/CD8 ratio was detected in grade II cases, early tumor stage, and non-TNBC. High CD4/CD8 ratio was also associated with absent lymphovascular and perineural invasion and related to cases with minor carcinoma in situ component. In concordance with our results, Wang et al.2017a also stated that increased CD4/CD8 ratio was related to small tumor size and early tumor stage favoring cases associated with lymphovascular invasion, and cases with advanced stage were higher than those in lymph node-negative cases, cases with no lymphovascular invasion, and early-stage cases, respectively. Seo et al., 2013 showed that high CD8+ T cell levels were strongly related to high histological grade and triple-negative molecular subtype.

Rathore et al., 2014 also found that higher numbers of CD8+ T cells were significantly associated with high grade, advanced stage and presence of lymphovascular invasion. Also, they said that the number of CD8+ T cells was not significantly related to the age of the studied cases. Matsumoto et al., 2016 reported that low CD8+ T cells were found in lower tumor grade, while high CD8+ T cells were found in higher grade. Khedr et al., 2016 said that higher tumor stage and TNBC were associated with increased CD8+ T cell number.

On the other hand, Matkowski et al., 2009 stated that CD8+ T cell numbers were higher in tumors without lymph node metastasis. They also found no relation between CD8+ T cells

better survival outcomes. Sheu et al., 2008 stated that low CD4/CD8 percentage was associated with lymphovascular invasion and lymph nodal metastasis.

On the other hand, Huang et al., 2015 found a significant association between CD4/CD8 ratio ≥1.2 with advanced tumor stage, large tumor size, and lymph nodal metastasis. Jafarian et al., 2018 found no significant association between CD4/CD8 ratio and tumor grade.

The different TILs results may be due to the use of variable TILs markers, variable cut-off values or varying location of TILs whether stromal, intra-tumoral, or both, as well as the different methods of assessing TILs (flow cytometry, tissue microarrays). We also found that there was strong negative correlation between PI3KCA expression and CD4/CD8 ratio, as high PI3KCA expression was associated with low CD4/CD8 ratio. In concordance with our results, Sobral-Leite et al., 2019 stated that PI3KCA strong expression was associated with high CD8+ T cells and low CD4/CD8 ratio.

CONCLUSIONS

Our results support that immunohistochemical expression of PI3KCA is related to poor clinicopathological parameters in studied IBC cases. As regards CD4 and CD8 expressions, there is a significant relation between low CD4 and high CD8 positive T-cells and unfavorable clinicopathological parameters.

A high CD4/CD8 ratio is associated with favorable clinicopathological parameters in the studied cases. Finally, there is a significant strong negative correlation between the expression of PI3KCA and CD4/CD8 ratio.

RECOMMENDATIONS AND LIMITATIONS OF THE STUDY

This study is limited by the small number of cases and lack of patients' follow-up data. So, we recommend conducting further studies on the expression of PI3KCA, CD4, and CD8 on a larger scale of cases including various histopathological subtypes of invasive breast carcinoma. Also, we recommend including the overall survival (OS) and disease-free survival (DFS) as main prognostic parameters and correlate the OS and DFS with the expression of PI3KCA, CD4, and CD8.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests

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