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

Mohamed Mohamed Eldeib, Safinaz Hamdy Elshorbagy, Asmaa Elsayed Bedeer and Nehal Abd El-Ghaffar Heabah

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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

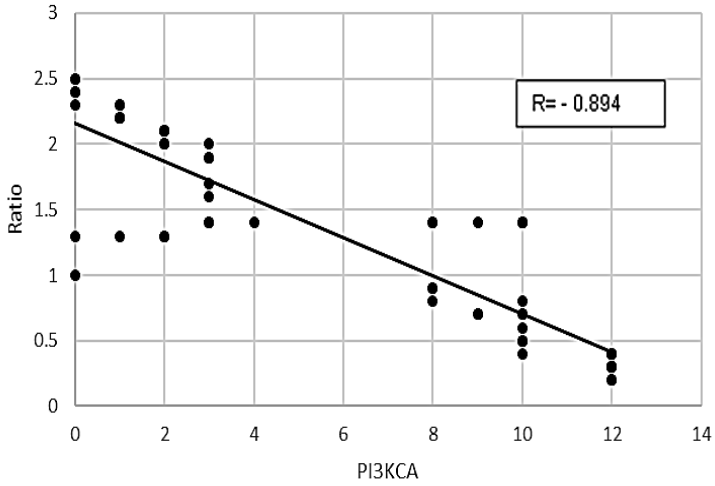

Figure 5. Correlation between PI3KCA and CD4/CD8 ratio in the studied cases

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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