Journal of Taibah University Medical Sciences (2024) 19(1), 99–105

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Expression of programmed death-ligand 1, IRF1 and CD8 T lymphocyte infiltration in a primary subset of breast cancer patients in Sudan

Shahenaz S. Salih, MSc [a,*], Mohammed S. Abdelaziz, Ph.D [a], Ibtehal M. Abdelhag, MSc [b] and Altaf S. Mosad, MSc [a]

[a] *Department of Histopathology and Cytology, Sudan University of Science and Technology, Khartoum, Sudan*
[b] *Department of Histopathology & Cytology, Omdurman Islamic University, Khartoum, Sudan*

Received 22 March 2023; revised 26 June 2023; accepted 28 August 2023; Available online 7 September 2023

الملخص

أهداف البحث: تهدف هذه الدراسة إلى الكشف عن تعبير بروتين ليجند 1 المبرمج للموت في أنسجة سرطان الثدي، مرتبطة بتعبيرات متنوعة لحالة الإستروجين، بالإضافة إلى تعبير عامل تنظيم الأنترفيرون الأول وتسلل اللمفاويات تي القاتلة المتعددة باستخدام المناعة الكيميائية. كما حاولنا الكشف عن العلاقة بين تعبير ليجند 1 المبرمج للموت، ومؤشر تكاثر الخلايا (مؤشر كي-67) ، ومدى تورط العقد اللمفاوية.

طرق البحث: تم جمع 150 كتلة ثدي مثبتة بالفورمالين ومدمجة بالبارافين من المختبر الصحي العام الوطني من النساء السودانيات. تم تعريض الكتل المثبتة بالفورمالين والمدمجة بالبارافين للكشف عن الأجسام المضادة/الأنتيجين باستخدام المناعة الكيميائية للأجسام المضادة لليجند 1 المبرمج للموت، وعامل تنظيم الأنترفيرون الأول، واللمفاويات تي القاتلة المتعددة، كل هذا تم تحليله بالإضافة إلى البيانات المستخرجة من السجلات لحالة الإستروجين، ومؤشر كي-67، وحالة العقد اللمفاوية.

النتائج: أظهرت تحليلات المناعة الكيميائية وجود علاقة هامة بين ليجند 1 المبرمج للموت واللمفاويات تي القاتلة المتعددة (بي = 0.010)، بالإضافة إلى ذلك، عكست الاختبارات الانحدارية قدرة عامل تنظيم الأنترفيرون الأول على زيادة تعبير ليجند 1 المبرمج للموت في الحالات التي تعبر عن عامل تنظيم الأنترفيرون الأول مرتين أكثر من الحالات التي تعاني من نقص في عامل تنظيم الأنترفيرون الأول (درجة الاحتمال = 2.441 بي = 0.035). النتائج التي توصلنا إليها، من جهة أخرى، أشارت إلى أن ليجند 1 المبرمج للموت له تأثير على تكاثر الخلايا كما ينعكس في مؤشر كي-67. تم استخدام اختبار تي المستقل، وكانت درجات كي-67 الأعلى تحدث بشكل متكرر بين الجماعات الإيجابية لليجند 1 المبرمج للموت أكثر من الأجزاء النقيضة السالبة (تي = 2.608 بي = 0.014). أظهرت العلاقة بين ليجند 1 المبرمج للموت وحالة الإستروجين أنها تحدث بشكل معاكس، حيث أظهرت الأورام الإيجابية للإستروجين تعبير سلبي لليجند 1 المبرمج للموت والعكس صحيح (بي = 0.04). علاوة على ذلك، حاولنا التحقيق في القيمة التنبؤية لليجند 1 المبرمج للموت من خلال النظر في الرابط بين تعبيره وانتشار العقد اللمفاوية بشكل متغير مع الخلايا السرطانية. توصلنا إلى أنه لا توجد علاقة ذات دلالة إحصائية بينهما.

الاستنتاجات: ظهر تعبير ليجند 1 المبرمج للموت، وتسلل اللمفاويات تي القاتلة المتعددة، وتعبيرات عامل تنظيم الأنترفيرون الأول، ليكونوا اللوحة البيولوجية الأكثر فعالية لتقديم مرضى سرطان الثدي للعلاج المناعي مع احتمالات عالية للرد الجيد بالأخذ في الاعتبار حالة الإستروجين، حيث تعبر عن الاستجابات السفلى لهذه المواد المثبطة للدورات الحيوية.

الكلمات المفتاحية: سرطان الثدي؛ تسلل اللمفاويات تي القاتلة المتعددة؛ الإستروجين؛ العلاج المناعي؛ عامل تنظيم الأنترفيرون الأول؛ ليجند 1 المبرمج للموت

Abstract

Objectives: This study aimed to investigate the protein expression of programmed death ligand 1 (PD-L1) in breast cancer (BC) tissues and link this data with estrogen status, the expression of interferon regulatory factor1 (IRF-1), and CD8+T lymphocyte infiltration by immunohistochemistry (IHC). We also attempted to identify the association between PD-L1 expression, the cell

* Corresponding address: Lecturer- Histopathology & Cytology, Sudan University of Sciences and Technology, Sudan.
E-mails: Shahinaznour86@gmail.com (S.S. Salih), mohamedsiddig88@gmail.com (M.S. Abdelaziz), altaftaaha12@gmail.com (A.S. Mosad)

Peer review under responsibility of Taibah University.
This project is under fund and support from Deanship of Scientific Resaerch-Sudan University of Science and Technology- Ministry of Higher Education & Scientific Research.

Production and hosting by Elsevier

 https://doi.org/10.1016/j.jtumed.2023.08.006

proliferation index marker (Ki67), and lymph node involvement.

Methods: One hundred and fifty formalin-fixed and paraffin-embedded (FFPE) blocks of breast tissue were acquired from Sudanese females *via* The National Public Health Laboratory. FFPE blocks were subjected to antigen/antibody detection by IHC with antibodies raised against PD-L1, IRF1, and CD8. These data were analyzed alongside data extracted from medical records relating to estrogen receptor (ER) status, Ki67 index, and lymph node (LN) status.

Results: IHC analysis revealed a significant association between PD-L1 and CD8 ($p = 0.010$). In addition, regression analysis indicated the ability of IRF1 to induce PD-L1 expression levels in IRF1-positive cases that were two-fold higher than IRF1-deficient cases (odds ratio [OR]: 2.441 $p = 0.035$). Analysis also suggested that PD-L1 exerts impact on cell proliferation, as reflected by the Ki67 index. An independent t test showed that higher Ki67 scores were more frequent among PD-L1-positive patients than in PD-L1-negative patients ($t = 2.608$ $p = 0.014$). There was an inverse association between PD-L1 and ER status; ER-positive tumors exhibited negative PD-L1 expression and *vice versa* ($p = 0.04$). Furthermore, we investigated the prognostic value of PD-L1 by evaluating the association between PD-L1 and LNs dispersed variably with tumor cells; there was no statistically significant relationship between these factors ($p > 0.05$).

Conclusion: The expression of PD-L1 and IRF-1, along with the infiltration of CD8, represents a potent panel of biomarkers with which to identify BC patients with the highest probabilities of achieving an excellent response to immune therapy, particularly when taking ER status into account, as ER expression levels are known to be high when immune checkpoint blockers (ICBs) generate a poor response.

Keywords: Breast cancer; CD8 infiltration; ER; Immunotherapy; IRF1; PD-L1

Introduction

Breast cancer (BC) is the most common cancer in women, and recent therapeutic advancements have led to the development of individualized and targeted medicines that halt disease progression and prolong the lives of patients.[1] Cancer immunotherapy, particularly the use of immune checkpoint blockers (ICBs), has emerged as a crucial area of research, with programmed death ligand 1 (PD-L1) garnering significant levels of research attention due to its association with different subsets.[2]

However, immune modulation has shown limited efficacy in patients with hormone receptor-positive BC, with significant responses primarily observed in cases of triple-negative breast cancer (TNBC). In TNBC, single-agent checkpoint inhibition yielded a response rate of approximately 5−24%, while patients with estrogen receptor (ER)-positive breast cancer showed an overall response rate of 12% or less.[2−6] Therefore, the PD-1/PD-L1 axis is considered an important target for breast cancer immunotherapy.[7] Increased levels of PD-L1 expression have been detected in inflammatory breast cancers (IBCs), ER-negative breast cancer, PR-negative breast cancer, basal tumors, and triple-negative breast cancer.[8]

In addition, survival patterns are known to vary depending on the type of immune infiltration and ER status. Tumors with low ER receptor expression but a high infiltration of CD8+ T lymphocytes and activated memory T cells are associated with a lower probability of relapse, whereas in ER-positive tumors, the presence of macrophages is correlated with a poor prognosis.[9]

Tumor growth creates an inflammatory environment that attracts a variety of immune cells, including CD8-cytotoxic lymphocytes. These lymphocytes play a key role in the expression of PD-L1, and this expression is drived by the release of interferon-gamma (IFN-γ) [10] from activated lymphocytes, such as CD8 and CD4 T cells and natural killer (NK) cells.[11]

IFN-γ-stimulated cells express high levels of interferon regulatory factor-1 (IRF-1), a potential transcription factor responsible for PD-L1 expression (IRF-1).[12] Moreover, the involvement of IFN-γ in the expression of PD-L1 is triggered by activation of the JAK/STAT1 pathway, thus leading to the release of IRF-1.[13−15]

IRF-1, a potent transcription factor, binds to the promoter region of PD-L1, thus resulting in mRNA synthesis and subsequent protein expression in the A549 human lung carcinoma cell line within 45 min of treatment with IFN-γ.[10] In addition, the JAK/STAT1/IRF-1 pathway has been correlated with prognosis and the levels of immune infiltration levels in breast cancer. However, there is a need to describe the tumor microenvironment fingerprint of breast cancer and investigate whether ER status affects this signaling cascade (15).

This study aimed to use immunohistochemistry (IHC) to investigate the expression levels of PD-L1 and IRF-1, along with the infiltration of CD8 lymphocytes, in cases of breast cancer with a known ER receptor status. In addition, we attempted to shed light on the impact of PD-L1 expression on cell proliferation, as reflected by the Ki67 index. Furthermore, we attempted to verify the potential of PD-L1 to enhance metastasis by studying its association with LNs.

Materials and Methods

This was a retrospective case study conducted in the National Public Health Laboratory, Khartoum, Sudan, between January 2019 and December 2021.

In total, 150 formalin-fixed and paraffin-embedded (FFPE) breast tissue blocks were acquired from Sudanese females who visited medical facilities and were diagnosed with breast cancer. The blocks were sectioned into 3 μm thickness ribbons and stained with Hematoxylin and Eosin. Additional sections were used for subsequent IHC tests with

an anti-CD8 mouse monoclonal antibody (Dako 32-M4), an anti-PD-L1mouse monoclonal antibody (73-10, Abcam, UK), and an anti-IRF-1 rabbit polyclonal antibody (A15842, AbClonal). Positive PD-L1 expression in the cytoplasm and/or cellular membrane was evaluated and correlated with previously available data relating to lymph node metastasis and Ki67 scores.

Immunohistochemistry

The positivity of PD-L1 was determined by a system that quantified the proportion and intensity of positivity using the histo-scoring system (H-score), a system that classifies cells by staining intensity and cellular density. Using this system, expression levels were classified into two groups according to a cut-off point of 100: scores of 0−99 represented negative expression while scores of 100−300 represented positive expression.[16] Scoring was assessed virtually using QuPath 0.3.2 software. Qu-Path0.3.2: https://qupath.github.io.

Samples were screened and relevant fields were captured by a Leica microscope; then, the images (or events) were imported into Qu-path0.3.2 software which sorted the stained cells according to DAB intensities (+1, +2, and +3); then, the software calculated the H-score for each event.

For CD8 T cell scoring, five randomly selected fields were scored; cells with a score of up to 25 were classified as +, those with a score of 26−50 were classified as ++, and those with a score >51 were classified as +++. A score of 25 represented low TILs infiltration while a score of >25 was considered as high TILs infiltration.

IRF-1 staining was qualitatively validated as either positive or negative. In addition, data relating to ER status, Ki67, and LN metastasis were collected from the primary medical records accompanying each case.

Statistical tests

Data were analyzed by SPSS-version 20 (IBM, New York, United States), including cross-tabulation, regression, independent T-tests, and one-way analysis of variation (ANOVA). In all analyses, a two-tailed P value < 0.05 was deemed statistically significant.

In this study, we aimed to identify the pattern of association between PD-L1 expression and the ER receptor. In addition, we hypothesized that mean PD-L1 scores were variably distributed between ER ± BC cases; for these analyses, we used one-way ANOVA.

With regards to immune cell infiltration into the tumor milieu, we investigated the association between CD8 T-lymphocytes and IRF-1 levels, ER status, Ki67 index and PD-L1; for these tests, we used Pearson's correlation with a significance value < 0.05.

In addition, the effect of IRF1 on PD-L1 expression was validated by a binary regression test with 95% confidence intervals (CIs). When EXP (B) or the odds ratio (OR) was <1, then an increase in the variable corresponded to a reduction in the odds of the event's occurrence, and *vice versa*.

Next, we ascertained whether Ki67 scores would vary among PD-L1 groups (+/−); we hypothesized that PD-L1 expression would increase cell proliferation indices (as reflected by Ki67 scores) and accelerate tumor growth, whereas PD-L1-deficient tumors would predominantly exhibit low Ki67 scores. To test this hypothesis, we performed an independent sample T-test, estimated the mean difference (M) with a 95% CI, and classified the tumors into two groups based on PD-L1 expression verified against Ki67 levels.

Finally, we investigated nodal involvement with tumor cells to identify further relationships that might link PD-L1 expression to the prognosis of disease. This relationship was statistically verified by cross-tabulation (the X^2 test) on 70 instances with comprehensive descriptions of nodal status; relevant clinical data were extracted from patient medical records.

Results

Of the 150 breast cancer cases; 85 patients were negative for estrogen receptor (56.7%) while 65 patients were positive (43.3%). Forty-three of the 150 patients showed positive PD-L1(CD274) expression in their tumor cells (28.7%; 43/150). In addition, we identified an association between these two markers in that there was a significant negative correlation between ER and PD-L1 expression, thus highlighting the possible antagonizing effect of upregulation of the *ESR1* gene on the expression of PD-L1 within the ER+ subset of BC patients. Table 1 shows the distribution and correlations between PD-L1 and ER expression. Figure 1 presents optical and density differences in PD-L1 expression according to different ER ± BC subtypes.

Moreover, we identified a significant difference in the distribution of PD-L1 scores between ER ± BC patients ($p = 0.026$). Mean difference analysis showed that the lower the ER expression, the higher the PD-L1 expression; this may point towards the antagonizing effects of ER on PD-L1. Figure 2 shows the negative association between ER status and PD-L1 scores. It is apparent that the PD-L1 score was reduced in ER-positive cases. The highest PD-L1 scores were detected in negative ER cases while the lowest PD-L1 scores were detected in positive ER cases.

With regards to the associations between CD8 and other biomarkers, we found that CD8 infiltration, IRF-1 expression, and PD-L1 expression, were in consensus, while ER exhibited a non-significant negative correlation. Ki67 presented with a non-significant association with lymphocytic infiltration. Table 2 presents the correlations between CD8 and these markers.

A binary regression test was used to investigate the effect of IRF1 on PD-L1 expression. We found that patients who were positive for IRF1 were 2.441-fold more likely to express PD-L1 than patients who were negative for IRF1, with a 95% CI of 1.063−5.603. These findings are shown in Table 3.

Table 1: Association between the ER subset and PD-L1 in BC.

		PD-L1		P-value
		Negative	Positive	
ER	Negative	55 (64.7%)	30 (35.3%)	0.040
	Positive	52 (80%)	13 (20%)	
	Total	107 (71.3%)	43 (28.7%)	
Spearman's roh		−0.168		

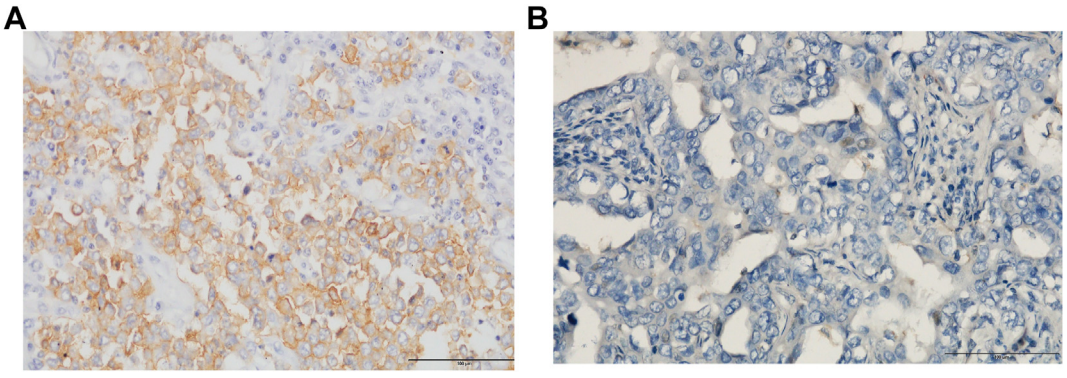

Figure 1: Expression of PD-L1 in Different Subsets of Breast Cancer according to Estrogen Receptor Status. A: PD-L1 expression in ER-negative Breast Cancer. B: PD-L1 expression in ER-positive Breast Cancer.

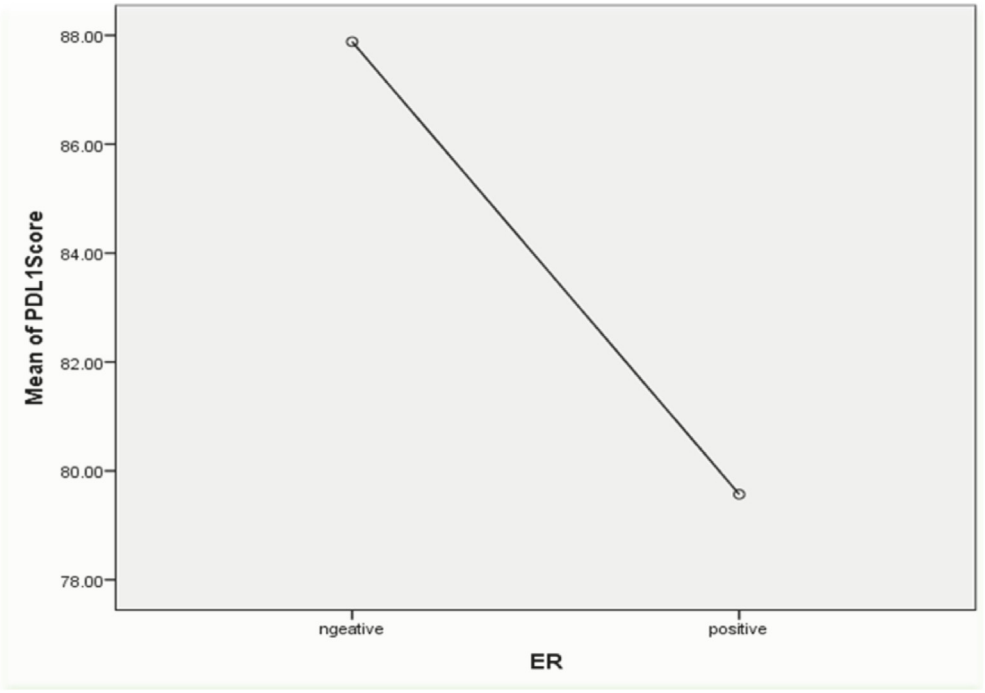

Figure 2: Reduction in the Mean PD-L1 Scores in the ER-positive Subset of BC Patients when Compared ER-negative Patients.

Table 2: Correlations between CD8 and PD-L1, IRF-1, ER and Ki67.

		IRF1	PD-l1	ER	Ki67
CD8	Spearman's-rho Correlation	0.299[b]	0.209[a]	−0.039	0.110
	Significance (2-tailed)	0.005	0.010	0.632	0.549
	Number	*150*	*150*	*150*	*32*

[a] Correlation is significant at the 0.05 level (2-tailed).
[b] Correlation is significant at the 0.01 level (2-tailed).

Table 3: IRF1 as a risk factor for PD-L1 expression.

Variable	EXP(B). Or (Odd ratio)	95% CI		P-value
		Lower	Upper	
IRF1	2.441	1.063	5.603	0.035

EXP(B): Exponential Value or the Odds Ratio; CI: Confidence Interval.

With regards to the mean difference in the distribution of Ki67 scores among PD-L1 groups, we identified a significant difference in the mean scores of Ki67 in the PD-L1-negative group of tumors when compared to PD-L1-positive tumors ($p = 0.014$); the t value was −2.608, thus implying that the mean Ki67 score in the PD-L1-positive group was 2.608-fold higher than that in the PD-L1-negative group. Thus, tumor populations lacking PD-L1 expression had lower Ki67 scores than the PD-L1-positive group. Table 4 shows Ki67 scores according to PD-L1 groups.

In the context of lymph node status and PD-L1 expression, the highest expression rates of PD-L1 were detected in 11 nodes with free metastasis; there was frequent (but non-significant) reduction in PD-L1 expression when nodes were more involved with tumor cells. Thus, there was no

Table 4: Mean Ki67 scores among PD-L1-negative/positive groups.

	PD-L1	Number	M	SD	t	df	P-value
Ki67	Negative	27	22.2	21.0	- 2.608	30	0.014
	Positive	5	48.0	14.8			

M (Mean), SD (standard deviation), t (the computed test statistic), *df* (degrees of freedom).

Table 5: Distribution of PD-L1 expression according to nodal status in breast cancer.

			PD-L1		Total	P value
			Negative	Positive		
LN	N0	Count	10	11	21	0.13
		% Within LN	47.6%	52.4%	100.0%	
	N1	Count	18	5	23	
		% Within LN	78.3%	21.7%	100.0%	
	N2	Count	9	4	13	
		% Within LN	69.2%	30.8%	100.0%	
	N3	Count	10	3	13	
		% Within LN	76.9%	23.1%	100.0%	
Total		Count	47	23	70	
		% Within LN	67.1%	32.9%	100.0%	

N0: No regional lymph node metastasis.
N1: Metastases in 1–3 axillary lymph nodes.
N2: Metastases in 4–9 axillary lymph nodes.
N3: Metastases in 10 or more axillary lymph nodes.

significant association between PD-L1 expression and nodal involvement. Table 5 shows the distribution of PD-L1 expression according to nodal status. Table 5 shows the distribution of PD-L1 expression between lymph nodes.

Discussion

Breast cancer (BC) is a leading cause of cancer-related deaths in women worldwide. The classification of different subtypes of invasive breast cancer are defined by expression levels of the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor (HER2), and Ki67 levels.[17]

In this study, we compared PD-L1 expression in ER-positive patients with ER-negative breast cancer patients and evaluated the correlation between dual expression. We found that ER expression was inversely associated with PD-L1 expression, thus indicating the antagonizing effect of ER expression on the upregulation of CD274. These findings are consistent with the findings of a previous study conducted by Shuai et al., who reported that a high degree of estrogen receptor expression reduced PD-1/PD-L1 expression and CD8+ T cell infiltration by reducing Th17 cell infiltration and IL-17 signaling transduction.[17]

PD-L1 expression is regulated by numerous signaling pathways *via* transcription and mRNA synthesis; the transcription factors IRF1 and IFN have been identified as the primary drivers of PD-L1 expression.[18] When considering the cross talk between IRF-1 and PD-L1, we detected rhythmic co-expression in the tumor milieu; this finding was consistent with previous findings reported by Yan et al., who found that IFN-gamma upregulated IRF-1 which subsequently increased the expression levels of PD-L1 mRNA and protein in both mouse and human hepatocellular carcinoma (HCC). Although the pivotal role of IRF-1 is to upregulate PD-L1, IRF-1 can also prevent IRF-2 from binding to the interferon regulatory element IRE promoter element in PD-L1, thus increasing regulatory activity of the PD-L1 pathway and generating therapeutic effects in HCC.[19]

Similar expression patterns were previously reported by Shao et al., who reported the potential of CD8 cells to reduce tumor growth in IRF1-deficient tumor cells in MC38 and CT26 colon carcinoma and a mouse model of B16 melanoma. IRF1-deficient tumor cells lost their ability to upregulate PD-L1 and showed no expression *in vitro* or *in vivo*, making them more vulnerable to T cell-mediated cell death; the induced restoration of PD-L1 expression rescued this situation and restored tumor growth.[18,20]

Usually, patients with PD-L1-positive CD8+ T cells and/or tumors with a high mutational burden are considered suitable candidates for PD-1/PD-L1-targeted therapy.[7] Since CD8 plays a crucial role in responses to ICB, most probably by controlling the expression of PD-L1, we attempted to explore the association between CD8 and other biomarkers, including IRF1, ER, and Ki67. Pooled data extracted from 1084 studies from the cBioPortal-TCGA and the PanCancer Atlas[21,22] were used to investigate the correlations between CD8, IRF1, Ki67, and ER mRNA. Analysis detected a significant positive correlation between CD8 and IRF1 ($p = 0.01$); this was in agreement with our present findings. The same data repository identified a significant negative correlation between CD8 and ER; a similar result was reported by Shuai et al. in 2020 who detected the high expression of CD8 in cases of ER-negative BC with advanced grades, a feature that contributed to high survival.[17] Our analysis also identified a negative correlation between CD8 and ER status, although this was not significant; this may be due to the small sample size in our study.

A previous study by Spathas et al. investigated the linkage of TILs to clinical outcomes in BC and found that the infiltration of CD8 was correlated to a higher Ki67 index.[23,24] Our present analysis found no evidence to support this correlation; this contradiction might be justified by the small number of samples with Ki67 data that were available for analysis.

In an attempt to investigate the variation in proliferation index according to different PD-L1 expression levels in BC tissues, we detected a significant association and correlation between PD-L1 expression and the Ki67 index, as well as a significant and variable distribution of Ki67 scores among PD-L1-positive and PD-L1-negative samples. Furthermore, we determined that tumors with prominent PD-L1 expression exhibited high levels of proliferation and *vice versa*, thus indicating that PD-L1 expression can exacerbate tumor growth, thus necessitating the use of ICB regimens. Our findings concur with those of Evangelou et al.[24]; furthermore, Rubino et al. reported that patients with tumors that are Ki67+/PD-L1+ have a lower overall survival rate when compared to patients with other tumors with variable Ki67/PD-L1 expression levels.[25]

With regards to nodal involvement, tumor cells and PD-L1 expression, we found no association between the number of nodes infiltrated with cancer cells and PD-L1; this finding did not agree with the previous findings of Yuan

et al., who reported that PD-1/PD-L1 positivity in metastatic lymph nodes was correlated with a poor prognosis, including a high Ki-67 index, a higher TNM stage, numerous metastatic lymph nodes, and a high histological grade.[24,26,27]

Conclusion

To the best of our knowledge, this is the first study to report PD-L1 expression levels in Sudanese women with breast cancer. We focused on a local population in order to identify the best candidates who might benefit from ICB therapy whenever it is accessible.

Based on the findings presented herein, we realize that the primary subsets of breast cancer with prominent estrogen levels may limit the efficacy and potential of ICBs, particularly those targeting PD-L1, simply because estrogen tends to antagonize the upregulation of PD-L1. On the other hand, the tumor milieus characterized by an abundance of CD8 cells, which subsequently triggers the expression of IRF1 and PD-L1, appear to be sufficient to assign patients for ICB therapy, particularly when the patient is deficient of ER but has a high mitotic index.

It is very common for primary subsets of BC patients to have a good prognosis, but it is also very common for these primary subsets to change drastically and aggressively over very short periods of time. This may be underlined by PD-L1 expression, which on the one hand would facilitate tumor cells to evade immune surveillance and maintain their mitotic activity; however, on the other hand, this may increase the mutational burden of the disease. Further research with larger sample sizes needs to investigate the specific associations between PD-L1 and lymph nodes. In addition, based on what we have observed in the present research, molecular studies targeting PD-L1 mutational levels are required as some tumors were negative for PD-L1 despite being infiltrated by CD8 and being positive for IRF1.

Source of funding

This Project was funded by the Ministry of High Education 2019 to the Deanship of Scientific Research, Sudan University of Sciences & Technology.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The Sudan University of Science and Technology's Institutional Ethics Committee gave its approval for this research (reference number: DSR-IEC-05-08). Since many patients had died and suspected survivors were uncontactable, it was difficult to obtain their consent. Hence to protect patient privacy, all samples and medical data utilized in this study have been securely anonymized.

Authors' contribution

SSS: Conceptualization, collected research materials, conducted research, performed laboratory experiments, analysed data, and wrote the original draft. MSA: Supervision, manuscript reviewing and editing, logistic support, and gave final approval of the manuscript draft to be published. IMA: participated in data acquisition and organization., provided research material and logistic support. ASM.: reviewed the draft manuscript, conducted statistical analysis and corrected the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Acknowledgements

We would like to acknowledge the Ministry of High Education; the National Public Health Laboratory; Dr. Alsaddiq Gassom and Dr. Sawsan Aldaif Centre from Neurosciences Ibrahaim Malik Hospital; Dr. Noha Elemam from SIMR-Sharjah University, and Dr. Fatima Magzoub, Dr. Aliaa, Dr. Wafaa Abdallah, Dr. Mariam Abbas Ibrahim, Dr. Mohamed Ahmed Salih, and Dr. Ahmed Muhktar for their support and cooperation.

References

1. Stovgaard ES, Dyhl-Polk A, Roslind A, Balslev E, Nielsen D. PD-L1 expression in breast cancer: expression in subtypes and prognostic significance: a systematic review. **Breast Cancer Res Treat 2019**; 174(3): 571–584.
2. Cejuela M, Vethencourt A, Pernas S. Immune checkpoint inhibitors and novel immunotherapy approaches for breast cancer. **Curr Oncol Rep 2022**; 24(12): 1801–1819.
3. Terranova-Barberio M, Pawlowska N, Dhawan M, Moasser M, Chien AJ, Melisko ME, et al. Exhausted T cell signature predicts immunotherapy response in ER-positive breast cancer. **Nat Commun 2020**; 11(1): 3584.
4. Adams S, Loi S, Toppmeyer D, Cescon DW, De Laurentiis M, Nanda R, et al. Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: cohort B of the phase II KEYNOTE-086 study. **Ann Oncol 2019**; 30(3): 405–411.
5. Emens LA, Cruz C, Eder JP, Braiteh F, Chung C, Tolaney SM, et al. Long-term clinical outcomes and biomarker analyses of atezolizumab therapy for patients with metastatic triple-negative breast cancer: a phase 1 study. **JAMA Oncol 2019**; 5(1): 74–82.
6. Rugo HS, Delord J-P, Im S-A, Ott PA, Piha-Paul SA, Bedard PL, et al. Safety and antitumor activity of pembrolizumab in patients with estrogen receptor–positive/human epidermal growth factor receptor 2–negative advanced breast cancer. **Clin Cancer Res 2018**; 24(12): 2804–2811.
7. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. **Nat Rev Cancer 2012**; 12(4): 252–264.
8. Bertucci F, Finetti P, Birnbaum D, Mamessier E. The PD1/PDL1 axis, a promising therapeutic target in aggressive breast cancers. **OncoImmunology 2016**; 5(3):e1085148.
9. Ali HR, Chlon L, Pharoah PDP, Markowetz F, Caldas C. Patterns of immune infiltration in breast cancer and their clinical implications: a gene-expression-based retrospective study. **PLoS Med 2016**; 13(12):e1002194.
10. Lee S-J, Jang B-C, Lee S-W, Yang Y-I, Suh S-I, Park Y-M, et al. Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-γ-induced upregulation of B7-H1 (CD274). **FEBS (Fed Eur Biochem Soc) Lett 2006**; 580(3): 755–762.
11. Burke JD, Young HA. IFN-Γ: a cytokine at the right time, is in the right place. **Semin Immunol 2019**; 43:101280.

12. Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN-γ in tumor progression and regression: a review. **Biomark Res 2020**; 8(1): 49.
13. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. **Cell 2017**; 168(4): 707−723.
14. Yan Y, Zheng L, Du Q, Geller DA. Interferon-γ/IRF-1 pathway regulatory mechanisms of PD-L1 expression and relevance for immune checkpoint blockade in hepatocellular carcinoma (HCC). **Oncotarget 2021**; 12(23): 2316−2317.
15. Moon JW, Kong S-K, Kim BS, Kim HJ, Lim H, Noh K, et al. IFNγ induces PD-L1 overexpression by JAK2/STAT1/IRF-1 signaling in EBV-positive gastric carcinoma. **Sci Rep 2017**; 7(1):17810.
16. Li Z, Dong P, Ren M, Song Y, Qian X, Yang Y, et al. PD-L1 expression is associated with tumor FOXP3(+) regulatory T-cell infiltration of breast cancer and poor prognosis of patient. **J Cancer 2016**; 7(7): 784−793.
17. Shuai C, Yang X, Pan H, Han W. Estrogen receptor down-regulates expression of PD-1/PD-L1 and infiltration of CD8+ T cells by inhibiting IL-17 signaling transduction in breast cancer. **Front Oncol 2020**; 10.
18. Shao L, Hou W, Scharping NE, Vendetti FP, Srivastava R, Roy CN, et al. IRF1 inhibits antitumor immunity through the upregulation of PD-L1 in the tumor cell. **Cancer Immunol Res 2019**; 7(8): 1258−1266.
19. Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. **Immunity 2018**; 48(3): 434−452.
20. Deng M, Li SH, Fu X, Yan XP, Chen J, Qiu YD, et al. Relationship between PD-L1 expression, CD8+ T-cell infiltration and prognosis in intrahepatic cholangiocarcinoma patients. **Cancer Cell Int 2021**; 21(1): 371.
21. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. **Cancer Discov 2012**; 2(5): 401−404.
22. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. **Sci Signal 2013**; 6(269): pl1.
23. Spathas N, Goussia AC, Koliou G-A, Gogas H, Zagouri F, Batistatou A, et al. Association between CD8+ tumor infiltrating lymphocytes and the clinical outcome of patients with operable breast cancer treated with adjuvant dose-dense chemotherapy—a 10 Year follow-up report of a Hellenic cooperative oncology group observational study. **Cancers 2022**; 14(22): 5635.
24. Evangelou Z, Papoudou-Bai A, Karpathiou G, Kourea H, Kamina S, Goussia A, et al. PD-L1 expression and tumor-infiltrating lymphocytes in breast cancer: clinicopathological analysis in women younger than 40 Years old. **In Vivo 2020**; 34: 639−647.
25. Rubino S, Kim Y, Zhou J, Dhilon J, Li R, Spiess P, et al. Positive Ki-67 and PD-L1 expression in post-neoadjuvant chemotherapy muscle-invasive bladder cancer is associated with shorter overall survival: a retrospective study. **World J Urol 2021**; 39(5): 1539−1547.
26. Yuan C, Liu Z, Yu Q, Wang X, Bian M, Yu Z, et al. Expression of PD-1/PD-L1 in primary breast tumours and metastatic axillary lymph nodes and its correlation with clinicopathological parameters. **Sci Rep 2019**; 9(1):14356.
27. Boman C, Zerdes I, Mårtensson K, Bergh J, Foukakis T, Valachis A, et al. Discordance of PD-L1 status between primary and metastatic breast cancer: a systematic review and meta-analysis. **Cancer Treat Rev 2021**; 99:102257.

How to cite this article: Salih SS, Abdelaziz MS, Abdelhag IM, Mosad AS. Expression of programmed death-ligand 1, IRF1 and CD8 T lymphocyte infiltration in a primary subset of breast cancer patients in Sudan. J Taibah Univ Med Sc 2024;19(1):99−105.