Research Article

Immunohistochemical Expression of PDL1 in Invasive Ductal Carcinoma of the Breast

Nehad M. Reda, Nisreen A. Abd El Gaber, Maram A. Mohammedan and Ola N. Mohammed.
Department of Pathology, Faculty of Medicine, Minia University, Egypt.

Abstract
Background: Breast cancer is the most common diagnosed cancer among females and the leading cause of cancer deaths. Immunohistochemical expression of PDL1 has been associated with bad prognosis in various malignancies including breast cancer. **Methods:** Immunohistochemical staining of PDL1 was performed on 60 tissue specimens of invasive ductal carcinoma by using the avidin biotin-peroxidase complex method with diaminobenizidine (DAB) chromogen detection system. **Results:** High expression of PDL1 was detected in tumor cells in 51.7% of cases. High expression was statistically significant with high tumor grade ($p<0.001$), LVI ($p=0.002$), infiltrated tumor margins ($p=0.039$), LN metastasis ($p=0.013$), ER- status ($p=0.012$), PR- status ($p=0.002$) and high Ki-67 expression ($p=0.031$). **Conclusion:** High PDL1 expression in tumor cells was associated with poor prognostic factors as high tumor grade, LVI, infiltrated tumor margins, LN metastasis and high Ki-67 expression. Overexpression of PDL1 is related to bad outcome of invasive ductal carcinoma cases.
Key words: PDL1; Invasive ductal carcinoma; Immunohistochemistry.

Introduction
Breast cancer (BC) is the most common diagnosed cancer and the leading cause of cancer deaths in women. In 2018, about 2 million new cases were diagnosed worldwide, accounting for almost 15% of all cancer deaths among women and 627.000 deaths were reported from BC (Bray et al., 2018).

In Egypt, BC is the most common malignant tumor among females accounting for 32.04% of diagnosed cancer cases. Its frequency of distribution is 38.7% in Upper Egypt followed by 33.8% in Lower Egypt then 26.8% in Middle Egypt (Ibrahim et al., 2014).

PDL1 is an immunoglobulin-like type I transmembrane glycoprotein. This is one of B7 family proteins that acts as a ligand for programmed death 1(PD-1). PDL1 is expressed on short arm of chromosome 9p24 and its molecular weight is approximately 40 kilodalton (Lin et al., 2008). PDL1 contains two anti-parallel β sandwich immunoglobulin super-family domains; domain 1 (D1) and domain 2 (D2) from N'- to C'-terminus joined by a short linker (Chen et al., 2010).

PDL1 expression was observed in different solid malignant tumors including bronchogenic, papillary thyroid, gastric, colorectal, hepato-cellular, renal cell and testicular carci-nomas with its relation to metastasis and bad prognosis (Chowdhury et al., 2015).

For BC, the expression data described were highly variable in different tumor subtypes. Studies pointed out that PDL1 up-regulation was associated with a better prognosis and response to chemotherapy (Sabatier et al., 2015; Baptista et al., 2016). However, other studies reported that the expression of PDL1 was associated with poor-prognostic clinic-pathological features and worse OS (Zhang et al., 2017; Li et al., 2018).

Material and Methods
1. Tissue specimens
This present study included 60 cases of breast invasive ductal carcinoma. Paraffin blocks were retrived from the archive of Pathology Department, Minia university Hospital in the period from December 2015 to April 2018. All specimens were obtained by modified radical mastectomy.

2. Immunohistochemistry

Monoclonal rabbit antibody, 7ml, ready to use, catalogue number: API 3171 AA, Clone: CAL10, Lab Vision Laboratories. For negative control the primary antibody was replaced with PBS, sections of lung adenocarcinoma and/or tonsillar tissue were used as positive control.

3. Scoring of Immunostaining

Histochemical score (H score) was used after assessment of staining intensity and percentage of stained tumor cells to produce a final score ranging from 0 to 300 by summation of individual H scores for each intensity (1x % cells of score1+2 x % cells of score 2+ 3 x % cells of score 3). PDL1 expression was categorized into two groups according to the H-score, using a cutoff score of ≥ 100 (H-score 0-99=low expression, 100-300=high expression) (Muenst et al., 2014; Li et al., 2016).

4. Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS software version 20). Raw data were compiled and range of various features. The Chi- square and Fisher's exact tests were used to compare categorical features. P value of < 0.05 was considered significant.

Results

1-Clinicopathological Features

Information regarding clinicopathological data of the cases was listed in **Table (1).**

Clinicopathological data		No.	(%)
Age (median in years)	<48	29	48.3%
	≥48	31	51.7%
Tumo size (cm)	<2cm	12	20%
	≥2cm	48	80%
mor focality	Unifocal	48	80%
	Multifocal	12	20%
Tumor grade	Grade I	4	6.6%
	Grade II	28	46.7%
	Grade III	28	46.7%
LVI	Absent	40	66.7%
	Present	20	33.3%
Tumor margins	Free	47	78.3%
	Infiltrated	13	21.7%
LN metastasis	Negative	20	33.3%
	1-3nodes	30	50%
	≥4 nodes	10	16.7%
Tumor stage	Stage I	15	25%
	Stage II	24	40%
	Stage III	20	33.3%
	Stage IV	1	1.7%
ER status	Negative	42	70%
	Positive	18	30%
PR	Negative	39	65%
	Positive	21	35%
HER2 status	Negative	50	83.3%
	Positive	10	16.7%
Ki- 67 sta	Low expression	19	31.7%
	High expression	41	68.3%

LVI: lymphovascular invasion, ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal receptor2 .

2- The association between PDL1 immunostaining in tumor cells and clinicopathological data

The association between PDL1 expression in tumor cells and clinicopathological data was summarized in **Table (2)**:

Clinicopathological data	Total 60 (100%)	Low expression 29 (48.3%)	High expression 31 (51.7%)	P value
Age (median in years)				
<48	29 (48.3%)	11 (37.9%)	18 (62.1%)	
≥48	31 (51.7%)	18 (58.1%)	13 (41.9%)	0.096
Tumor size (cm)				
<2cm	12 (25%)	7 (58.3%)	5 (41.7%)	
≥2cm	48 (75%)	22(45.8%)	26 (54.2%)	0.326
Tumor focality				
Unifocal	48 (80%)	26 (54.2%)	22 (45.8%)	
Multifocal	12 (20%)	3 (25%)	9 (75%)	0.067
Tumor grade				
Grade I	4 (6.6%)	4 (100%)	0(0%)	
Grade II	28 (46.7%)	21 (75%)	7 (25%)	
Grade III	28(46.7%)	4 (14.3%)	24 (85.7%)	<0.001*
Necrosis				
Absent	43(71.7%)	22 (51.2%)	21 (48.8%)	
Present	17 (28.3%)	7 (41.2%)	10 (58.8%)	0.341
LVI				
Absent	40 (66.7%)	25 (62.5%)	15 (37.5%)	
Present	20 (33.3%)	4 (20%)	16 (80%)	0.002*
Tumor margins				
Free	47 (78.3%)	26 (55.3%)	21 (44.7%)	
Infiltrated	13 (21.7%)	3 (23.1%)	10 (76.9%)	0.039*
LN metastasis				
Negative	20 (33.3%)	5 (75%)	5 (25%)	
1-3 nodes	30 (50%)	11 (36.7%)	19 (63.3%)	
≥4 nodes	10 (16.7%)	3 (30%)	7 (70%)	0.013*
Tumor stage				
Stage I	15 (25%)	10 (66.7%)	5 (33.3%)	
Stage II	24 (40%)	11 (45.8%)	13 (54.2%)	
Stage III	20 (33.3%)	8 (40%)	12 (60%)	0.312
Stage IIII	1 (1.7%)	0 (0%)	1 (100%)	
ER status				
Negative	42 (70%)	16 (38.1%)	26 (61.9%)	
Positive	18 (30%)	13 (72.2%)	5 (27.8%)	0.012*
PR status				
Negative	39 (65%)	12 (30.8%)	27 (69.2%)	
Positive	21 (35%)	17 (81%)	4 (19%)	0.012*
HER2 status				
Negative	50(83.3%)	25(50%)	25(50%)	
Positive	10(16.7%)	4 (40%)	6 (60%)	0.841
Ki-67 status				
Low expression	19 (31.7%)	13 (68.4%)	6 (31.6%)	
High expression	41(68.3%)	16 (39%)	25(61%)	0.031*

We detected high PDL1expression in 51.7% and low expression in 48.3% of the studied cases. High expression was statistically significant with high tumor grade (p<0.001), LVI (p=0.002), infiltrated tumor margins (p=0.039), LN metastasis (p=0.013), ER- status (p=0.012), PR- status (p=0.002) and high Ki-67 expression (p=0.031).

Discussion

We detected high PDL1expression in 51.7% and low expression in 48.3% of the studied cases and this was in agreement with previous study (Adams et al., 2018) that reported high expression in 58% and low expression in 42% of cases. Also Park et al., (2016) showed high expression in 51.6% and low expression in 48.4% of the cases. On the other hand, high expression was found in 35.5% and low staining was found in 64.5% of cases due to different scoring system from the one used in our work (Botti et al., 2017).

The present study reported a statistically significant association between PDL1 expression and tumor grade (p<0.001); as high expression was observed more in grade III cases (85.7%). This finding was consistent with Li et al., (2016) who found high expression in 57% of grade III cases. On the contrary, Botti et al., (2017) observed high expression in 37.3% of grade III patients. This may be attributed to the different methods of scoring system and different cut off points chosen in that research.

Our study detected a statistically significant association between PDL1 expression and LVI (p=0.002). High PDL1 expression was seen in cases with LVI (80%) and this was in line with what was noted by Meng et al., (2018) who reported high PDL1 expression in 85% of cases. Meanwhile high PDL1 expression was in 18% of cases with LVI. Cases in that study were on regimen of chemotherapy that has influenced and changed the tumor microenvironment (Manson et al., 2019).

A statistically significant association was detected between PDL1 expression and tumor margin (p=0.039); high expression was assessed more in cases with infiltrated margin (76.9%) which was in agreement with Julia et al., (2017) who detected high expression in 60.5% of cases. On the other hand, Droeser et al., (2013) reported high PDL1 expression in 40% of cases with positive margins in colorectal cancer. This may be attributed to the different tissue used with different genetic component of tumor.

We found a statistically significant association between PDL1 expression and LN status (p=0.013); as high expression was detected more in cases with LN metastasis (65%). This finding was in agreement with Kitano et al., (2017) who showed high expression in 56% of LN positive cases. On the contrary to our results, high expression was seen in 22% of LN positive cases, which may be related to different population ethnicity as that study was done on Asian patients (Qin et al., 2015).

A statistically significant negative association was detected between PDL1 expression and ER status (p=0.012); as high expression of PDL1 was observed more in cases with ER- tumors (61.9%). This goes in line with Qin et al., (2015) and Wang et al., (2017) who detected high PDL1 expression in 53% and 78% of ER- cases respectively.

Also a statistically significant negative association was detected between PDL1 expression and PR status (p<0.001); as high expression was detected more in cases of PR- (69.2%). This was concordant with Li et al., (2016) and Kim et al., (2017) who detected high PDL1 expression in 51.9% and 55.3% of PR- cases respectively.

In the current study, a statistically significant association was observed between PDL1 expression and Ki-67 expression (p=0.013); high PDL1 expression was observed more in cases with high Ki-67 expression (61%) which was consistent with previous studies of Sabatier et al., (2015) which reported high PDL1 expression in 62% of cases with high Ki-67 expression. While Alves et al., (2019) didn't report high PDL1 expression in cases with high Ki-67 and that may be due to doing the research on metastatic cases with different gene mutation and tumour characteristics.

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