

## PD-1 / PD-L1 EXPRESSION IN INVASIVE BREAST CARCINOMA / CANCER

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### ABSTRACT

**Objective:** To investigate the immunohistochemical expression of PD-1 and PD-L1 in breast cancer cases and its correlation with clinicopathological factors.

**Study Design:** Cross sectional study.

**Place and Duration of Study:** Histopathology department, Armed Forces Institute of Pathology Rawalpindi, from Jan to Jun 2019.

**Methodology:** Sixty five cases of breast cancer were retrieved. Clinicopathological parameters like age, gender, tumor grade and receptor status were noted. Immunohistochemistry for PD-1 and PD-L1 was applied. The data was entered and statistical analysis was done using SPSS version 21. The associations between variables were found using the Fisher exact test.

**Results:** Sixty five cases of breast cancer were investigated from tumour registry. The sample included female patients, having mean age of  $50.86 \pm 11.1$  Years. Invasive mammary (ductal) carcinoma, NST was the most prevalent subtype 60 (92.3%) and most tumors were grade I/II 53(82%). PD-1 expression was seen in TIL's 31 (48%) and PD-L1 expression was observed in tumour cells 30 (46%). Expression of PD-1/PD-L1 expression was more common in premenopausal age group and grade I/II tumors. Among molecular subtypes, PD-L1 expression was detected in 11 (52%) TNBC, 5 (71%) in HR-/HER2+ and 12 (32)% in HR + HER2 - and PD-1 expression was observed in 13 (62%) TNBC, 5 (71%) in HR-/HER2+ and 13 (35%) in HR+ HER2-.

**Conclusion:** A large proportion of breast cancer cases show expression of PD-1 / PD-L1. Anti PD-1 and PD-L1 therapy may benefit these patients.

**Keywords:** Invasive breast cancer, Programmed cell death protein 1, Programmed death-ligand 1.

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### INTRODUCTION

Breast cancer has the highest prevalence among women all over the world. The world health organization (WHO) states that every year 2.1 million women are diagnosed with breast cancer and it is one of the key factors of cancer related mortality among women<sup>1-3</sup>. There are a variety of etiological agents responsible for the development of breast carcinoma, but Shen *et al* suggests that there are molecular alterations at the cellular level which are responsible for the disease. In our bodies, the immune system performs an important function in protecting ourselves from different diseases including cancer cells<sup>4</sup>. The immune suppression is known to be the trademarks of cancer. A variety of receptors and their ligands known as checkpoints regulates this process.

Checkpoints are proteins present on immune cells surface which are activated or inactivated to generate an immune response. The cancerous cells protect themselves from immune system by using these checkpoints. The PD-1 (programmed cell death-1) receptor is a checkpoint protein present on activated T cells and its ligand PD-L1 is present on some normal cells including nerves, muscles and also on the surface of cancer cells including tumour associated macrophages which are present in the tumour microenvironment and T cells. PD-L1 is the ligand for PD-1. When PD-L1 on the tumour cells surface binds to PD-1 receptor present on activated T cells it causes initiation of the PD-1/PD-L1 pathway which in return causes T cell inhibition and prevents cytotoxic T cells from attacking other cells in the body<sup>5</sup>. These deactivated T cells remain inhibited in the tumor microenvironment. This is one of the mechanism through which cancer cells escapes the immune system. The cancer cells have considerable

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quantity of PD-L1, and uses this mechanism to prevent themselves from immune attack<sup>6,7</sup>.

Targeted therapies against PD-1 or PD-L1 have recently been introduced and are in clinical trial<sup>8</sup>. The anti PD-1 / PD-L1 therapies comprise different monoclonal antibodies that are targeted against PD-1/PD-L1. These anti PD-1/anti PD-L1 antibodies block the binding of PD-1 receptor to its ligand PD-L1. This enhances the immune response and has an innovative effect on cancer treatment. Therefore, PD-L1 also serves as a prognostic marker and is used for assessing tumour response and may predict survival and prognosis. A number of studies have elaborated that the tumour that are PD-L1 positive show better tolerance to anti PD-1/PD-L1 antibodies than those of conventional therapeutic agents and show longer overall survival<sup>9</sup>. Keeping in view the nature of these checkpoint inhibitors, more studies are needed to check PD-L1 expression in various tumours and find its correlation with clinical consequences.

A variety of different tumours expresses PD-L1 expression including breast cancer. There are four different molecular subtypes of breast cancer. Among them the breast cancer cases that are triple negative (TNBC) are thought to be aggressive tumours having higher tumour grade and high risk of distant metastasis. New therapeutic agents are needed to improve the management of triple negative breast cancer. Previous studies suggest that PD-L1 is expressed in higher number of breast cancer cases that are triple negative as compared to other subtypes<sup>10</sup>. Therefore by blocking PD-1/PD-L1 signaling pathway, apoptosis can be induced and this may be used as a treatment against tumours which uses this pathway. The signaling pathway manifesting PD-1 / PD-L1 has been described in various tumours which includes malignant melanomas, cancers of colonic origin, lung carcinomas and ovarian cancers. Literature shows that Anti PD-1 antibodies have been used in the past to treat non-small cell lung carcinomas and malignant melanomas<sup>11</sup>. However, few studies have observed PD-1/PD-L1 expression in breast carcinoma. So we checked

expression of PD-1/PD-L1 in our population and its correlation with clinicopathological features and hormone receptor subtypes.

The means of detecting PD-L1 by immunohistochemistry and to be used as a biomarker for predicting response to therapy is under clinical trials. However, previous studies have demonstrated that this marker PD-L1 alone is not sufficient to predict that what type of patients will get benefit from anti PD-1/PD-L1 therapy<sup>12</sup>. Additional biomarkers which would demonstrate higher levels of PD-L1 in tumour may help in better selection of the patients for treatment with antibodies directed against PD-1 / PD-L1 proteins.

The purpose of this study was to check the expression of PD-1/PD-L1 in breast cancer patients and find its association with clinicopathological factors, as anti PD-1 / PD-L1 therapies are available and these patients might get benefit.

## METHODOLOGY

The present study was based on cross sectional design and it was conducted at the department of histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from January to June 2019. The study was conducted after getting permission from Institutional Review Board (IERB No. FC-HSP-15-17/Read-IRB/19/542). Sample size was calculated using WHO calculator by taking anticipated frequency at 13.5% and confidence interval limits at 8.5%. The cases were recovered from tumour registry which comprised on already pathologically diagnosed 65 patients of invasive breast cancer based purposive sampling technique. Two pathologists reviewed the cases and according to the WHO (2015) diagnostic criteria, confirmed the diagnosis. Association with clinicopathological parameters, which includes age, gender, pathological classification, and hormone receptor subtypes were noted. Paraffin embedded blocks were taken and the immunohistochemical staining was performed according to the companies' instructions. The tissue from normal tonsil was taken as control for PD-1 and tissue from human placenta was taken as a positive control for PD-L1. PD-1

staining was observed in the cell membranes of TILs and PD-L1 was identified in cell membranes of tumour cells. A single cell staining (1%) was considered as positive for both PD-1 and PDL-1. The data was entered and statistical analysis was done using SPSS version 21. The associations between variables were found using the Fisher exact test. A significance level of  $\leq 0.05$  was taken as statistically significant.

**RESULTS**

The sample comprises 65 cases. All the patients were female having mean age of  $50.86 \pm 11.1$  Years. Invasive mammary (ductal) carcinoma, NST was the most common subtype 60 (92.3%), followed by mixed invasive ductal and invasive lobular carcinoma 2 (3.1%), metaplastic carcinoma 1 (1.5%), pleomorphic lobular carcinoma with ductal carcinoma in situ 1 (1.5%) and Solid Papil-

lary Carcinoma 1 (1.5%). Most tumors were grade I/II 53(82%). A number of breast cancer cases showed expression of ER 37 (57%), PR 35 (54%), HER2 9 (14%), TNBC 21 (32%), HR-/HER2+ 7 (11%) and HR+/HER2- 37 (57%). PD-1 expression was seen in 31 (48%) TILs and PD-L1 expression was observed in 30 (46%) tumour cells. The expression of PD-1 was significantly correlated with PD-L1 ( $p < 0.01$ ) and the expression was more common in premenopausal age group and grade I/II tumors as displayed in table-I. PD-1 expression was more common in tumours which are ER negative 18 (64%), PR negative 19 (63%) and HER2 positive 5 (56%). Similarly PDL-1 expression was seen mostly in tumours that are ER negative 16 (57%), PR negative 16 (53%) and HER2 positive 7 (78%) as shown in table-II. Among molecular subtypes, PD-L1 expression was detected

**Table-I: Clinopathological parameters associated with PD-1/ PD-L1 expression.**

		No. of Patients (%)		No. of Patients (%)	
		PD-L1 Expression (n=65)		PD-1 Expression (n=65)	
		Positive (n=30)	Negative (n=35)	Positive (n=31)	Negative (n=34)
Age (Years)	$\leq 50$	18 (60)	18 (51)	18 (58)	18 (53)
	$> 50$	12 (40)	17 (47)	13 (42)	16 (47)
Grade	1 or 2	20 (67)	33 (94)	22 (71)	31 (91)
	3	10 (33)	2 (6)	9 (29)	3 (9)
Subtype	ER+	14 (47)	23 (66)	13 (42)	24 (71)
	ER-	16 (53)	12 (34)	18 (58)	10 (29)
	PR+	14 (47)	21 (60)	12 (39)	23 (68)
	PR-	16 (53)	14 (40)	19 (61)	11 (32)
	HER2+	7 (23)	2 (6)	5 (16)	4 (12)
	HER2-	23 (77)	33 (94)	26 (84)	30 (88)

**Table-II: Correlation of PD-1/ PD-L1 with hormone receptor studies.**

	ER		PR		HER2	
	Positive (n=37)	Negative (n=28)	Positive (n=35)	Negative (n=30)	Positive (n=9)	Negative (n=56)
PD-1+	13 (35%)	18 (64%)	12 (34%)	19 (63%)	5 (56%)	26 (46%)
PDL-1+	14 (38%)	16 (57%)	14 (40%)	16 (53%)	7 (78%)	23 (41%)
<i>p</i> -value	0.098		0.018		0.439	

**Table-III: Expression of PD-1/ PD-L1 according to molecular subtypes.**

		All (n=65)	TNBC (n=21)	HR-/ HER2+ (n=7)	HR+/ HER2- (n=37)
Median Age		50 (26-74)	52 (40-65)	46 (40-60)	50 (26-74)
<b>Patients, n (%)</b>					
Grade	1 or 2	53 (82)	15 (71)	5 (71)	33 (89)
	3	12 (19)	6 (29)	2 (29)	4 (11)
PD-1 in tumour infiltrating lymphocytes	Positive	31 (48)	13 (62)	5 (71)	13 (35)
	Negative	34 (52)	8 (38)	2 (29)	24 (65)
PD-L1 in tumour cells	Positive	30 (46)	11 (52)	5 (71)	12 (32)
	Negative	35 (54)	10 (48)	2 (29)	25 (68)

in 11 (52%) TNBC, 5 (71%) in HR-/HER2+ and 12 (32)% in HR+ HER2- and PD-1 expression was observed in 13 (62%) TNBC, 5 (71%) in HR-/HER2+ and 13 (35%) in HR+HER2 as revealed in table-III.

## DISCUSSION

The signaling pathway of PD-1/PD-L1 among various tumours is currently under focus these days. However, limited data is available regarding breast cancer in the recent years.

Previously the expression of PD-L1 was demonstrated in different tumours and a prior study by Wu, Wu, Li, Chai and Huang (2015) revealed that the positivity of PD-L1 was related with reduced overall survival of patients. They conducted a meta-analysis and suggested that expression of PD-L1 is associated with worse 3 years overall survival in solid tumors (95% confidence interval (CI)=1.60 to 3.70,  $p<0.0001$ )<sup>13</sup>. However, association of PDL-1 and prognosis of breast cancer patients remains uncertain. Qin *et al* (2015), Muenst *et al* (2014) and Li *et al* (2016) have reported that manifestation of PD-L1 was related with poor outcomes. They studied the expression of PD-L1 and FOXP3+ Treg infiltration and their results showed that both these immunohistochemical markers are positively associated with a high histological grade, negative ER and PR status and the intrinsic subtype of breast cancer<sup>14-16</sup>. On the other hand some studies have not yet confirmed these findings. Park *et al* investigated the relationship between TIL profiles for CD8+ and forkhead box P3- positive (FOXP3+) and showed that positive correlation between CD8+ TILs and FOXP3+ TILs. Although no association was found between FOXP3+TILs and PD-L1 expression, moreover they also showed that PD-L1 expression was more frequent in HR-positive breast cancer. Another study conducted by Baptista, Sarian, Derchain, Pinto and Vassallo (2016) showed that PD-L1 expression was significantly associated with better overall survival ( $p=0.04$ ) in breast cancer patients<sup>17,18</sup>.

The higher expression levels of PD1/PDL1 has been previously studied in various tumours.

A study by Velho *et al* suggested that PDL-1 was expressed in a Prostatic Carcinoma patients subgroup which are aggressive in nature. Their results showed that high PD-L1 levels are positively correlated with Gleason score 5 ( $p=0.004$ )<sup>19</sup>.

Mandalà *et al* analyzed the expression of PDL-1 in metastatic melanoma and came to the conclusion that PDL1 can predict response to treatment and showed that there was decreased risk of mortality rate by 53% in metastatic melanomas patients who have expressed PD-L1 proteins on the tumour cells and have received anti-PD-L1 antibodies<sup>20</sup>.

Teixidó *et al* has found better survival with anti PD-L1 therapy in patients of non-small cell carcinoma of lung and showed that response rate was 45.2%<sup>21</sup>.

FDA has permitted the use of targeted therapies against PD1/PDL-1 in various tumours including non-small cell carcinomas of lung, Hodgkins lymphoma, metastatic melanomas, renal cell carcinoma, bladder tumours, gastric cancers and hepatocellular carcinomas. PD-1/PDL1 expression has not been studied much in breast cancer<sup>22</sup>. In a study by Kitano *et al* showed that concurrent expression of PD-1/PD-L1 in breast cancer cases was 34% which is comparable to our results which show 48% expression of PD-1 and 46% expression with P-DL-1<sup>23</sup>. They also stated that these markers were related with higher histological grade of tumour and molecular subtype. Moreover, they also stated that the levels of PD-1/PD-L1 can be utilized to measure the response to therapy.

In this study, we examined PD-1 and PD-L1 protein expression in 65 cases of breast carcinoma. PD-1 was observed in TILs and the expression of PDL-1 was seen in tumour cells and we have seen their association with clinico-pathological factors and tumour subtypes. Our findings suggests that PD-1 was expressed in significant number of breast cancer cases and was considerably associated with PD-L1 expression in breast carcinoma ( $p<0.01$ ). We also found that PD-1/PDL-1 was most commonly expressed in tumours



that are estrogen and progesterone receptor negative. However it is mostly seen in tumours which are positive for Her 2 receptor. According to molecular subtypes PD-1/PDL-1 expression was most common in HR-/HER2+ subtype than in TNBC subtype. Our results are comparable to a study conducted by Lou *et al* which demonstrates that PDL-1 expression is seen in number of breast cancer cases 37.5%. They also stated that the expression of PDL-1 was mostly associated with ER, PR negative tumors but are high in those tumors that are HER-2 positive<sup>24</sup>.

A meta-analysis conducted by Beckers *et al* showed that the manifestation of PDL-1 can serve as a putative biomarker of response to PD1 therapy and is expressed very commonly in TNBC. Their results showed that basal like breast cancer subtypes mostly have higher expression levels compared to luminal subtype.

Another study by Soliman *et al* demonstrated that number of breast cancer cases express PDL-1 and it is more commonly seen in basal type breast cancer<sup>25</sup>.

Moreover, a meta-analysis carried out by Guo *et al* reported the manifestation of PD-L1 in breast carcinoma is linked with poor clinical and pathological factors such as high tumour grade, nodal metastasis and negative estrogen receptor status<sup>26</sup>.

The similarity of our results with prior studies indicates that PD-1/ PD-L1 is manifested in breast carcinoma and is mostly associated with estrogen and progesterone receptor negative tumours. However, our study has few limitations. It was performed on limited number of cases, secondly the breast carcinoma cases showing positive expression of PD-1 / PD-L1 was seen by applying immunohistochemical studies and not transcriptionally and we did not see the association with tumour stage and overall survival.

## CONCLUSION

A significant number of breast cancer cases expressed PD-1/PDL-1. The expression is most commonly seen in ER-/HER+ subtype. Targeted

therapy against PD-1/PD-L1 receptors might boost the immune system and eradication of breast cancer cells may be achieved. Additional data is required to discover the association of anti PD-1 / PDL-1 therapy in breast carcinoma.

## CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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