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Study of PD-L1 expression using laboratory developed manual test and its clinicopathological correlation in solid tumors – A two-year experience in a tertiary care cancer centre.

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Abstract

Background: Immune modulation therapies have seen an impressive growth over the last decade. Five PD-1/PD-L1 immuno therapies (atezolizumab, avelumab, durvalumab, nivolumab and pembrolizumab) have now been approved by the United States (US) Food and Drug Administration (FDA) and/ or Euro pean Medicines Agency (EMA).

Immuno his to chemistry (IHC) bio marker assays for respective PD-1/PD-L1 inhibitors were designed to estimate the percentage of T lymphocytes and tumor cells expressing this receptor or ligand. Our aim is to study PD - L1 expression in solid tumors using laboratory developed test and study its clinico patho logical cor relation over a period of two years. Also, to emphasize the use of low- cost, manual, laboratory-developed test (LDT) PD-L1 IHC assay using the easily available CAL 10 clone.

Method: The present study was conducted which in cluded 51 cases of various solid tumors which were examined for PD-L1 expression in tumor and immune cells by immuno his to chemistry (Laboratory Developed Test) during two-year period. PD-L1 positivity was defined as Combined Positive Score (CPS) greater than or equal to 1 and TPS greater than or equal to 1% of tumour cells. Demographic data, clinical features, patho logical findings were also noted.

Results: Out of total 51 cases, 26 (50.9%) showed PD-L1 positivity as per CPS and 21(41.2%) cases showed tumour cell positivity as per TPS. 57.5% of primary and 27.2% of metastatic cancers showed PD-L1 positivity. 68.7% (11/16) of Squamous cell carcinomas and 38.7% (12/31) of adeno carcinomas and 1 case each of Infit rating duct carcinoma, small cell carcinoma and endo metrioid carcinoma showed positivity. Out of all primary tumours, PDL1 positivity is seen mostly in well differenti ated tumours (54.5%), then moderately (27.3%) and

poorly differentiated tumours (18.2%). Highly positive cases (CPS >50 and TPS .50%) were of squamous cell carci noma (6 cases) and adeno carcinomas (3 cases). PD-L1 positivity also corre lated with high tumour infil trating lymphocytes (>50%).

Conclusion: PD-L1 expression has a male preponde rance with 19 positive cases and mean age 63.1 yrs. High PD-L1 was associated with lower tumour grade (6/8) cases and more with squamous cell carcinoma cases. Our study attempted to address an important issue i.e., utility of an economical laboratory-developed assay as an alter native to expensive auto mated commercial predictive IHC assay so that patient therapy remains un compro mised and affordable.

Keyword: PDL1, CPS, TPS, Immuno his to chemistry, Squamous cell carcinoma, Adenocarcinoma.

Introduction

Immune modulation therapies have seen an impressive growth over the last decade. PD-L1 typically expressed on the surface of healthy cells, binds PD-1 on printed cytotoxic T cells thereby inhibiting cell-mediated attack. Anti-PD-L1 (or anti PD-1) monoclonal antibodies inhibit PD-L1 binding to PD-1 and allow T cell activity at this immune check point [1,2].

Five PD-1/PD-L1 immuno therapies (atezolizumab, avelumab, durvalumab, nivolumab and pembrolizumab) have now been approved by the United States (US) Food and Drug Administration (FDA) and/ or Euro pean Medi cines Agency (EMA) [3]. Tumor expression of Prog rammed death-ligand 1(PD-L1) is an important pre dictive bio marker for treatment with immune checkpoint inhibitors (ICIs) [4,5].

Immuno his to chemistry (IHC) biomarker assays for respective PD-1/PD-L1 inhibitors were designed to screen for the presence of specific PD-1/PD-L1 epitopes as well as to estimate the percentage of T lymphocytes and tumor cells expressing this receptor or ligand. Some cancer cells have a high amount of PD-L1. This allows the cancer cells to 'trick' the immune system and avoid being attacked as foreign, harmful substances [6,7].

Immuno therapy is a therapy that boosts your immune system to help it recognize and fight cancer cells. PD-L1 is a trans membrane protein that down regulates immune responses through binding to its two receptors program med death-1 (PD-1) and B7-1 (CD80). PD-L1 is an immune check point protein that regulates the immune response to prevent excessive/ chronic auto immune in flammation [8,9].

Our aim is to study PD-L1 expression in solid tumors on immuno his to chemistry and its clinicopathological cor relation over a period of two years. Also to emphasize the use of low-cost, manual, laboratory-developed test (LDT) PD-L1 IHC assay using the easily available CAL 10 clone [6].

Materials and methods

The present study is conducted in Department of Patho logy and Onco pathology, Mahatma Gandhi Medical College and Hospital, Jaipur. Study population included 51 cases of various solid tumors which were examined for PD-L1 expression in tumor and associated immune cells by immuno his to chemistry for two years period. It is a Prospective, non – rando mized, observational study. Patient's demo graphic data, clinical features, patho logical findings and PD-L1 expression is noted in all cases.

PD-L1 positivity was defined as Combined Positive Score (CPS) greater than or equal to 1 and TPS as > 1% of tumour cells. Combined Positive Score (CPS) greater than or equal to 1, where CPS is the number of PD-L1 positive cells (tumour cells, lymphocytes and macro phages) divided by total number of viable tumour cells multiplied by 100. The Tumour Proportion Score (TPS)

is the percentage of viable tumour cells showing partial or complete membrane staining (greater than or equal to 1+) relative to all viable tumor cells present in the sample (positive and negative).

Positive PD-L1 staining/expression is defined as complete and/or partial, circumferential or linear plasma mem brane staining at any intensity that can be different iated from background and diffuse cytoplasmic staining.

IHC has done through Ultra Vision Hydrogen Peroxide Block, Ultra Vision Protein Block and primary antibody Amp lifer Quanto technique. Apply HRP Polymer Quanto and then DAB Quanto chromogen and DAB Quanto substrate. Counterstain and coverslip using a per manent mounting media on the slide. Rabbit anti-human PDL1 monoclonal antibody (CLONE CAL10) is used in our institute.

As per literature, CPS score of PDL1 positivity greater than or equal to 1 is considered in HNSCC and Gastric carcinoma and greater than or equal to 10 in oesophageal and TNBC cases while TPS is considered positive when greater than or equal to 1% in Lung cases.

Results and Discussion

Out of total 51 cases, 26(50.9%) showed PD-L1 positivity as per CPS. In the study, 21(41.2%) cases showed tumour cell positivity as per TPS. The study showed male predo minance with 19 positive cases and mean age 63.1 yrs. Most common age group having maxi mum number of positive cases was 51-60 years (9 cases) followed by 61-70 years age group.

The study showed 23 cases out of 40 primary (57.5%) and 3 cases out of 11 metastatic cancers (27.2%) showed PD-L1 positivity. 11 cases out of 16 squamous cell carci noma (68.7%) and 12 cases out of 31 adenocarcinomas (38.7%), 1 case each of Infiltrating duct carcinoma, small cell carcinoma and Endo Metroid carcinoma showed positivity. Out of all primary tumours PDL1 positivity is seen mostly in well differentiated tumours (54.5%), then moderately (27.3%) and poorly differentiated tumours (18.2%). 100% positivity were see in Oesophagus, Breast, Thyroid and Endometrium sites showed positivity in all cases followed by lung (85%) and then stomach (80%).

Most commonly CT and USG guided biopsies were taken for assessment of PDL1. Combined positive score (CPS) and Tumour proportion score (TPS) were also evaluated and correlated with clinicopathological para meters. CPS was < 1 in 24 cases, 1-10 in 10 cases, 11-49 in 8 cases and ≥ 50 in 9 cases. Rest cases showed no PD-L1 expression. TPS was <1% in 19 cases, 1-10% in 6 cases, 11-49% in 6 cases and ≥ 50% in 9 cases. Rest cases showed no PD-L1 expression. Highly positive cases (CPS >50 and TPS >50%) were of squamous cell carcinoma (6 cases) and adenocarcinomas (3 cases). In all positive squamous cell carcinomas cases, 54.5% cases showed high positivity. PD-L1 positivity also correlated with high tumour infiltrating lymphocytes (>50%). According to CPS scoring (≥1) in HNSCC and Gastric cases, 3 and 4 cases are positive for PDL1 respectively while in oesophageal and TNBC cases 1 case each was positive (CPS scoring ≥10). According to TPS scoring (≥1%) in Lung cases, 10 cases are positive for PDL1.

Discussion

In the present study, PD-L1 expression was seen more in males (76.4%) as compared to females (23.5%) with mean age of 62 yrs. Ur ska Janzic et al [7] (2017), showed that PD-L1expression more in male patients (63%) than in females (37%) with mean age of 62.7 yrs. Ekta Jain et al [8] (2021), showed that high PD-L1 expression was observed more prevalent in female patients (32.4%) and age of ≥ 60yrs. This is compatible with the present study.

PD-L1 expression positivity seen more in squamous cell carcinoma (62.5%) than adenocarcinoma (27.7%). Urska

Janzic et al [7] (2017), studied revealed that PD-L1 expression was significantly higher in tumor cells of squa mous cell carcinoma as compared to adeno carcinoma. This correlation is also seen in the present study. Yan Jin et al [9] (2019), showed that squamous cell carcinoma (27.4%) than adenocarcinoma (6.5%). This is compatible to our study. Ekta Jain et al [8] (2021), was observed that adeno carcinoma had more prevalent as compared to squamous cell carcinoma.

In the study, PD-L1 expression were found more in well differentiated (46.4%) and poorly differentiated tumors (37.5%) than moderately differentiated tumors (33.3%). Ekta Jain et al [8] (2021), was observed that poorly differ entiated had more prevalent than well and moderately differentiated tumors. Konrad Pawelczyk et al [10] (2019), showed high PD-L1 expression in higher-grade of malignancy, which is also seen in this study.

In the study, out of total 21 positive cases, 21 showed positivity in tumor (TPS) and inflammatory cells (CPS) while remaining 3 showed positivity in inflammatory cells only. Kohei Yamashita et al [11] (2020), was ob served that PD-L1 positivity were detected by TPS (20. 4%) and (71.7%) by CPS. This showed that CPS was a signifi cant independent factor to express PD-L1 positi vity than TPS. This is similar in the present study too.

Highly positive cases (CPS >50% and TPS >50%) were of squamous cell carcinoma (6 cases) and adeno carcino mas (3 cases). PD-L1 positivity also correlated with high tumour infiltrating lymphocytes (> 50%).

Tables and figures

Table 1: Showing distribution of total and positive cases (as per CPS) according to age group

Age group (years)	Total	Positive (%)	Highly positive
31-40	2	2 (100)	1
41-50	3	2 (66.6)	-
51-60	17	9 (52.9)	4
61-70	13	7 (53.8)	3
71-80	13	5 (38.5)	1
81-90	3	1(33.3)	-
Total	51	26 (50.9)	9

Graph 1: Showing distribution of total and positive cases according to gender

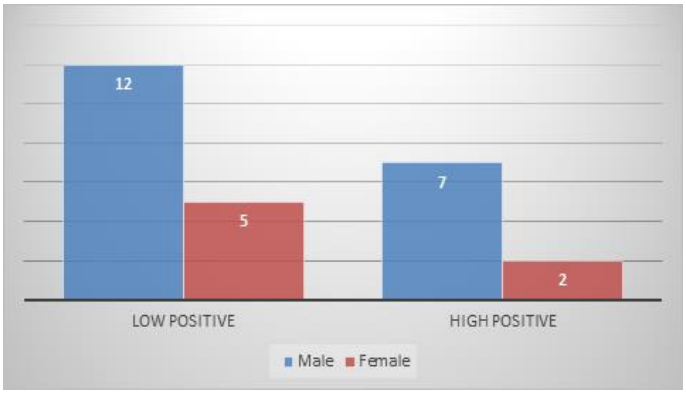


Table 2: Showing distribution of primary vs meta static tumors showing PD-L1 positivity.

Site	Low positive	High positive	Total positive	Negative	No. Of cases
Primary	15	8	23	17	40
Metastatic	2	1	3	8	11
Total no. Of cases	17	11	26	25	51

Graph 2: Showing different types of biopsy specimens.

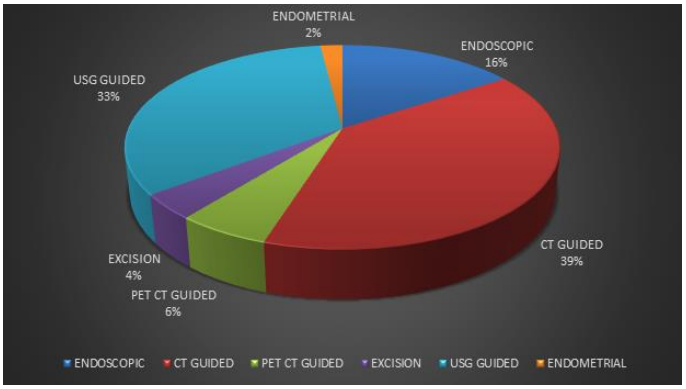


Table 3: Showing distribution of total and positive cases according to the site.

Site	Cases	Positive	%	High positive
Lung	20	10	50	7
Liver	7	3	42.8	1
Head and neck	5	3	60	-
Bone	4	0	0	-
Esophagus	1	1	100	1
Gall bladder	2	1	50	-
Breast	1	1	100	-
Lymph node	1	0	0	-
Stomach	5	4	80	-
Thyroid	1	1	100	-
Endometrium	1	1	100	-
Abdomen site (others)	3	1	33.3	-
Total	**51**	**26**	**50.1**	**9**

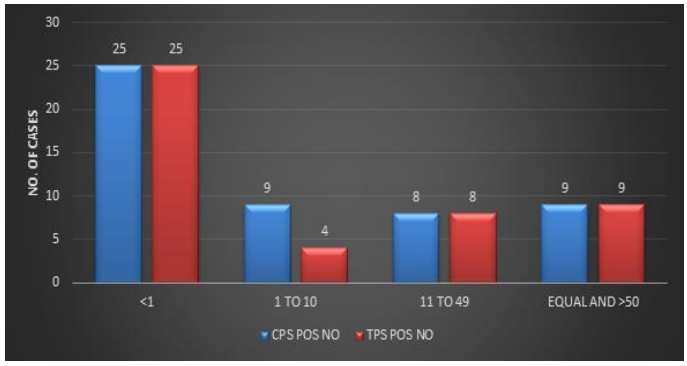
Table 4: Showing CPS and TPS score in primary tumours.

Positive	Well differentiated	Moderately differentiated	Poorly differentiated	Total
TPS	11	5	3	19
CPS	12	6	4	22

Table 5: Showing distribution of positive cases as per histopathological diagnosis.

Diagnosis types	Low positive	High positive	Positive	Negative	Total
Adenocarcinoma	9	3	12	19	31
Squamous cell carcinoma	5	6	11	5	16
Hepatcellular carcinoma	0	0	0	1	1
Small cell carcinoma	1	0	1	0	1
Infiltrating ductal carcinoma	1	0	1	0	1
Endometroid carcinoma	1	0	1	0	1
Total	17	9	26	25	51

Graph 3: Showing distribution of positive cases as per CPS and TPS score.



Graph 4: Showing distribution of positive case of TPS and CPS in patients as per site.

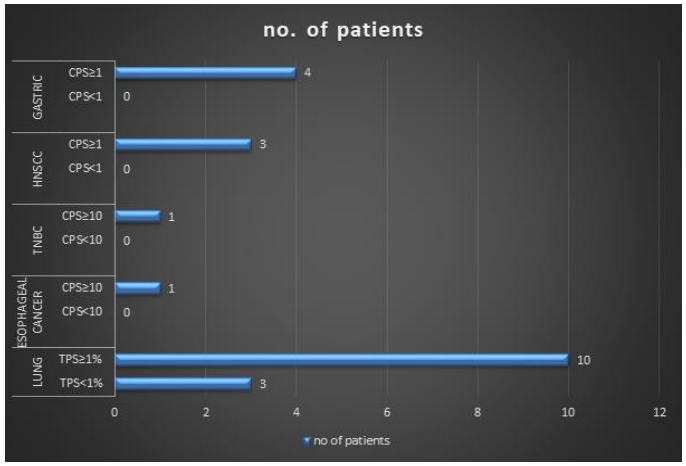


Figure 1: A-Case of well differentiated squamous cell carcinoma. B-IHC staining of the tumor in which only tumor cells are positive for PD-L1; while tumor immune cells are negative. CPS: 80. (master diagnostic a CAL10 anti-body)

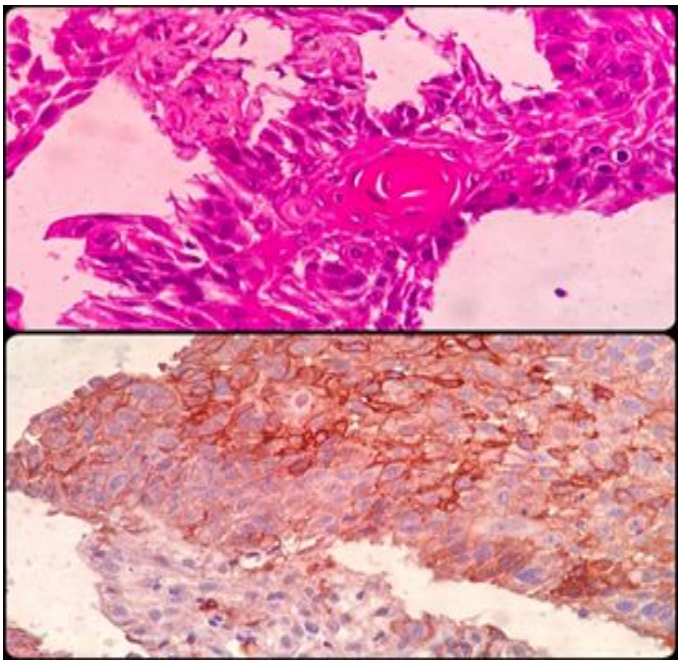


Figure 2: A-Case of Moderately differentiated adeno carcinoma. B-IHC staining of the tumor in which tumor cells are negative for PD-L1; while tumor immune cells are positive. CPS: 10. (Master diag nostic a CAL10 anti body

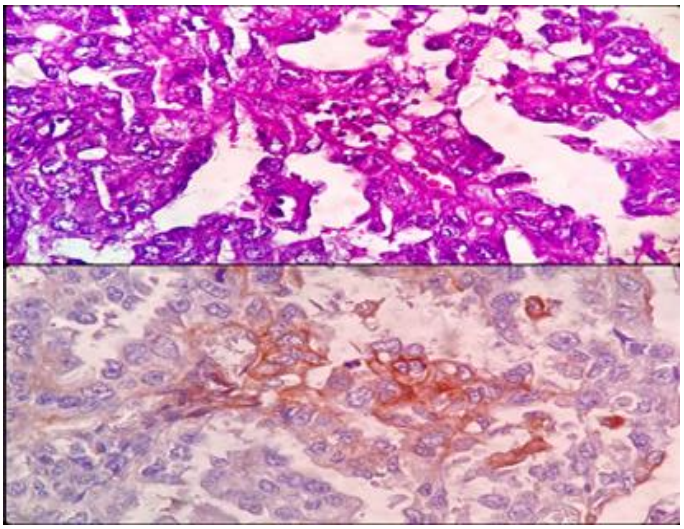


Figure 3: A-Case of poorly differentiated Endo Metroid carcinoma. B-IHC staining of the tumor in which tumor cells are positive for PD-L1; while tumor immune cells are negative. CPS: 20. (Master diagnostic a CAL10 antibody)

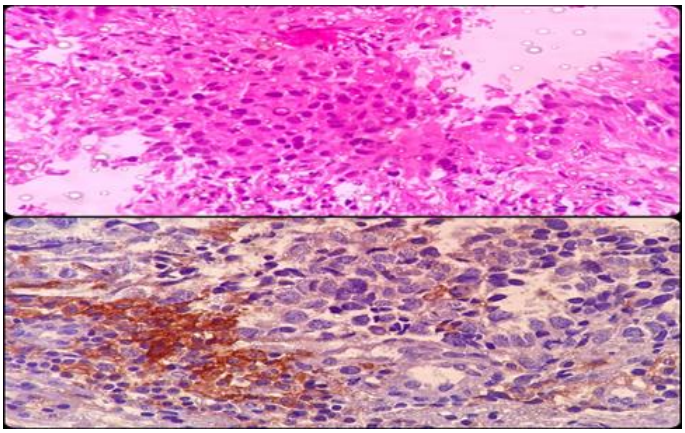
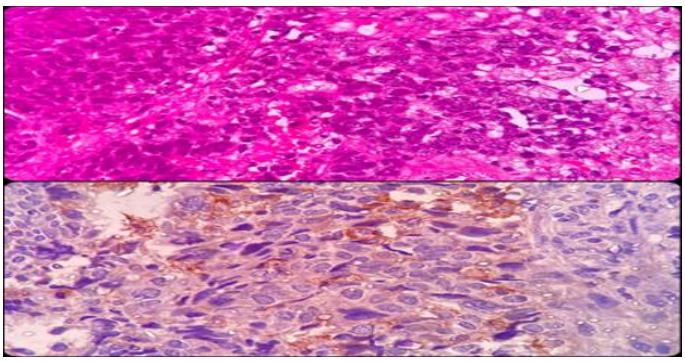


Figure 4: A-Case of poorly differentiated infiltrating duct carcinoma. B-IHC staining of the tumor in which tumor cells are negative for PD-L1; while tumor immune cells are positive. CPS: 35. (Master diagnostic a CAL10 anti body)



Conclusion

PD-L1 expression has a male preponderance with 19 positive cases and mean age 63.1 yrs. High PD-L1 was associated with lower tumor grade (6/8) cases and more with squamous cell carcinoma cases.

Our study attempted to address an important issue i.e., utility of an economical labo ratory - developed manual assay as an alternative to expensive auto mated commercial predictive IHC assay so that patient therapy remains uncompromised and afford able. However validation studies are needed for further analysing this issue.

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