Research Article

PD-L1 expression in gastric carcinoma – a biomarker for immunotherapy

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ABSTRACT

Introduction and Aim: Immunotherapy is now being widely used for targeted therapy in several cancers. The PD-1/ PD-L1 (programmed cell death receptor/programmed cell death ligand) axis inhibits the expansion of tumour specific T cells and prevents the immune response against tumours. PD-L1 expression is seen in many cancers including gastric carcinomas and anti PDL1 antibodies are being used to produce tumour regression. This study was done to evaluate PD-L1 expression in gastric carcinomas and correlate this with the different histological types, grades and stages of these tumours

Materials and Methods: In this study, 50 cases of gastric adenocarcinoma were analysed for PD-L1 expression using immunohistochemistry. PD-L1 expression was correlated with histopathological factors including histological type, differentiation, grade and stage using statistical methods.

Results: A positive PD-L1 expression was observed in 52% of the cases. A positive correlation between PD-L1 positivity and tumour depth to which the tumour was invading the stomach was observed. No correlation was noted with expression of PD-L1 and histological type, differentiation, histological grade and presence/absence of nodal metastasis.

Conclusion: PD-L1 expression was seen in more than half of the patients in our study. Hence it is suggested that anti PD-L1 inhibitors can be used as an adjunct in the treatment of gastric cancers, especially those with a greater depth of invasion.

Keywords: PD-L1; gastric cancer; checkpoint inhibitor; immunotherapy.

INTRODUCTION

Gastric cancer is a common cause of cancer that contributes to increased morbidity and deaths in the world. It is the fifth most frequently diagnosed cancer and the third most common cause of cancer related mortality after lung and colorectal cancer, being responsible for one in every 12 deaths globally. In India, it is the 4th most common cancer; third most common in men and the fifth most common in women(1,2). The National Cancer Registry Program of the Indian Council of Medical Research (ICMR) states that gastric cancer occupies the leading site (9.1%) in Chennai, the fourth leading site (6.4%) in Bangalore – both cities in South India and the fifth (5.4%) in Dibrugarh in the North Eastern part of India (3).

In localized gastric cancers, the standard treatment regimen is complete resection with a wide margin and systematic lymphadenectomy with or without perioperative chemotherapy. Since majority of the patients with gastric cancers present at an advanced stage, successful treatment is limited to only those patients detected at early stages. Usage of platinum and 5-fluorouracil (5-FU) together for treating the late stages of gastric carcinoma, has not given a significant survival benefit (4,5).

Newer treatment strategies such as immunotherapy have shown promising results in the field of oncology (6). The infiltration of tumours by activated T cells (CD8+) is the most important part of the primary host immune response against solid tumours and this correlates with better survival. Cancer immunotherapy uses the host's natural defence mechanism to amplify the antitumour immunity for a stronger antitumour effect. At present, treatment protocols for the immune therapy of cancers are adoptive cellular immunotherapy, checkpoint inhibitors, and therapeutic cancer vaccines (7-9). Cancers escape immune destruction through numerous mechanisms, one of which includes the activation of checkpoints which are inhibitory elements that limit self-damaging autoimmunity in normal individuals. Few of the immune checkpoints activated by tumours to create an immunosuppressive environment are PD-1, CTLA-4, LAG-3, TIM- 3. Thus, inhibition of these cancer activated checkpoints would pave way for the therapy of these tumours and this has been used with significant effects in the treatment of cancers like melanoma, lung and kidney cancers (10).

Programmed cell death ligand 1 (PD-L1) is a key immunoregulatory molecule which interacts with PD-1, a receptor present on the surface of the T cells.

This interaction suppresses the CD8 cytotoxic immune response to tumours. PD-L1 expression has been recognized in several solid tumours, such as renal cell carcinoma, breast cancer, pancreatic cancer, colorectal cancer and oesophageal cancer (11,12).

Multiple studies on gastric carcinoma in which immunohistochemistry (IHC) for PD-L1 was done, showed varying rates of PD-L1 expression, ranging from 5.1% to 65%. Several clinical trials have shown that usage of inhibitors of PD-1 and PD-L1 for treatment of gastric carcinomas have increased the overall survival rates in advanced gastric cancers, especially when combined with chemotherapy (13).

In our study, PD-L1 immunohistochemical expression was studied on tissues from surgical biopsies and specimens of gastric carcinoma and correlated with the histopathological features of the tumours.

MATERIALS AND METHODS

A total of 50 cases of gastric carcinoma presenting over a period of two years from 2018 to 2020 were included in the study. This consisted of both biopsies and resected specimens of gastric adenocarcinoma of both sexes and all ages. Gastric biopsies with insufficient material, benign and malignant gastric disorders other than adenocarcinoma and cases with preoperative chemotherapy were excluded.

Ethical clearance was obtained from the Scientific Review Board and Institutional Ethical Committee.
Paraffin blocks of cases of gastric adenocarcinoma which had been placed for a sufficient period in 10% formalin of neutral pH and processed were retrieved and sections were cut serially at a thickness of 4 microns. Hematoxylin and eosin (H &E) dyes were used to stain the sections.

Details of the histological type, differentiation, grade, depth of invasion, lymphovascular and perineural invasion and nodal involvement were obtained by perusing the H & E sections and suitable blocks were selected for immunohistochemical staining.
The tumours were classified histologically into two types, namely, intestinal and diffuse using the commonly used Lauren classification. Grading of the tumour based on the differentiation and descriptions of specialized tumour types were obtained from the WHO classification of Digestive System Tumours, 2019. The well differentiated and moderately differentiated gastric adenocarcinomas were grouped under low grade and the poorly differentiated tumours were grouped under high grade tumours (WHO 2019). Tumour staging had been done based on the American Joint Committee on Cancer (AJCC) (8^{th} edition, 2017) Tumor/node/metastasis (TNM) classification and staging system for gastric cancer.

Sections of 4µm thickness were cut from representative paraffin blocks of gastric biopsies and resected specimens of gastric carcinoma. These were affixed to slides coated with Poly L Lysine. The primary antibody used for PD-L1 detection was Tinto PD-L1, Clone-RBT-PD-L1(22C3) of BioSB. The secondary antibody used was Master Polymer Plus Detection System of Master Diagnostica. Sections from paraffin blocks of placental and tonsillar tissue were processed and stained along with the test samples to provide the positive control for PD-L1 staining
All well-preserved tumour areas were examined at low magnification and the overall areas of PD-L1 stained and non-stained tumour cells were evaluated. At least a 100 well preserved tumour cells had to be present in the PD-L1 section for adequacy. The number of the above cells was determined. Any convincing partial or complete linear membrane staining of viable tumour cells that was perceived as distinct from cytoplasmic staining was considered PD-L1 positive and was included in the scoring. Any convincing membrane and/or cytoplasmic staining of lymphocytes and macrophages, mononuclear inflammatory cells (MICs) within tumour nests and/or adjacent supporting stroma was considered PD-L1 positive and was included in the scoring. The number of PD-L1 stained cells namely tumour cells, lymphocytes and macrophages was determined and the combined positive score (CPS) was calculated.

CPS = Number of PD-L1 stained cells (tumour cells, lymphocytes, macrophages) /Total number of viable tumour cells x 100

A CPS $\geq$1 was considered as PD-L1 positive and CPS <1 as PD-L1 negative.

Statistical analysis was done with SPSS version 22.0 software. The variables were tabulated as frequencies and percentages. The data between the groups were compared using Chi-square test. For all statistical tests, a $p < 0.05$ was considered a significant difference (14,15).

RESULTS

Out of the 50 cases, 32 were biopsies and 18 were resected specimens. Among the 50 cases of the study, 26 (52%) of the cases showed PD-L1 positivity and 24 (48%) of the cases were PD-L1 negative. Most of the intestinal type tumours (60%) showed PD-L1 positivity and most of the diffuse tumours (58.3%) showed absence of PD-L1 positivity. The only mixed type carcinoma showed PD-L1 positivity. In our study, 61.5% of the tubular (intestinal) type, 50% of poorly cohesive type, showed PD-L1 positivity while none of the signet ring cell types and mucinous carcinomas were positive. (Fig. 1) The medullary and the mixed carcinoma types also showed PD-L1 positivity (Fig. 2).

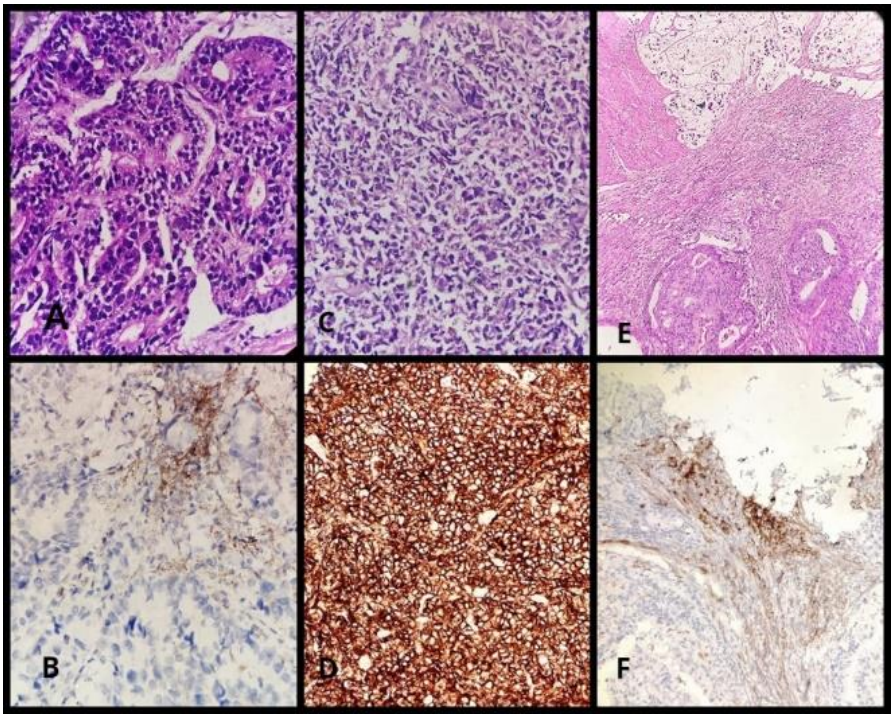

Fig.1: A) Intestinal type gastric adenocarcinoma (H&E ; x40). B) positive PD-L1 staining in intestinal type gastric adenocarcinoma in (IHC; x40). C) Diffuse type gastric adenocarcinoma (H&E; x40). D) positive PD-L1 staining in diffuse type gastric adenocarcinoma in (IHC; x10). E) Mixed type gastric adenocarcinoma (H&E; x10). F) positive PD-L1 staining in mixed type gastric adenocarcinoma in (IHC; x10).

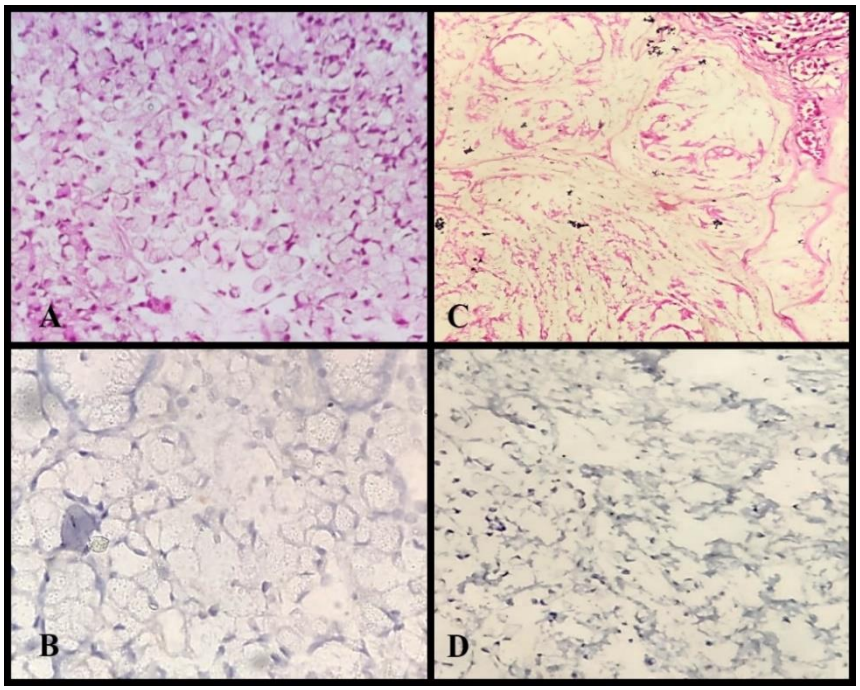

Fig. 2: A) Signet ring cell gastric carcinoma (H&E;x10) . B) PD-L1 negative staining in Signet ring cell gastric carcinoma (IHC ;x40). C) Mucinous gastric carcinoma (H & E; x10) D) PD-L1 negative staining in mucinous gastric carcinoma (IHC; x40).

In the present study, 60% of the low grade tumours and 56% of the high grade Tumors showed PD-L1 positivity (p=0.258). Among all the biopsies and resected specimens, 13(56.5%) moderately differentiated tumors, 11(44%) poorly differentiated tumors and 2(100%) well differentiated tumours showed positive expression for PD-L1 staining. 10 (43.5%) moderately differentiated tumors and 14(56%) poorly differentiated tumors showed negative expression for PD-L1. Among the resected

specimens, 1(25%) pT2 stage tumor, 1(11.1%) pT3 stage tumour, 2(66.66%) pT4a stage tumours and both (100%) the pT4b stage tumours showed PD-L1 positivity and this correlation showed statistical significance (p=0.014; Table 1).

Table I: Correlation between tumour invasion depth and PD-L1

Tumour stage		**PD-L1**		**Total (P value = 0.014)**
		Positive	**Negative**	
TNM Type T	pT2	1(25%)	3(75%)	4
	pT3	1(11.1%)	8(88.8%)	9
	pT4a	2(66.6%)	1(33.3%)	3
	pT4b	2(100%)	0	2
Total	Count	6	12	18

On comparing the nodal involvement with PD-L1 expression, 1(25%) pN3a stage tumour, 2(28.6%) of the pN2 stage tumours, 1(33.33%) pN1 stage tumour and both the pN0 tumours showed PD-L1 positivity. The pNx stage tumours were PD-L1 negative. (p value = 0.268)

DISCUSSION

Despite efforts to improve treatment, including usage of combination strategies with chemotherapy and targeted therapy, the majority of patients of gastric carcinoma eventually progress and the prognosis remains poor. This necessitated the need for strategies such as immunotherapy (16-18).

In this study, PD-L1 positivity in the tumours was noted in majority of the patients (52%) which is in accordance with other studies conducted worldwide (Table 2). An extensive search of literature failed to show similar studies in the Indian subcontinent and we believe this could be a forerunner of many more to come.

Table 2: Comparison of PD-L1 positivity with previous studies that used CPS scoring

No.	**Reference**	**Country**	**PD-L1 positivity % (CPS scoring)**
1	Kim *et al.,* (17)	Korea	60.5
2	Park *et al.,* (18)	Korea	57.8
3	Kim *et al.,* (19)	Korea	84.6
4	Liu (20)	Korea	59.3
5	Present study	India	52

Majority of the tubular (intestinal) carcinomas, half the poorly cohesive carcinomas and the mixed and medullary carcinomas showed positive PD-L1 expression. None of the signet ring cell carcinomas or the mucinous carcinomas showed positive expression for PD-L1. In the studies of Liu *et al.,* and Huang *et al.,* the tumours showed a lesser PD-L1 positivity in signet ring cell tumours when compared to other histological types. These findings are unlike those of signet ring cell carcinomas of the colorectum wherein PD-L1 positivity has been found to be increased. However Huang *et al.,* has reported an increased PD-L1 expression in peritoneal recurrences of advanced gastric cancer with signet ring cells as well as signet ring cell carcinomas that have metastasized to the liver or ovary (20,21). Currently there is no data regarding the PD-L1 expression in mucinous gastric adenocarcinomas and studies are being conducted for the same (22).

In our study PD-L1 positivity was more frequently present in the low grade tumours than the high grade ones. Although the frequency of PD-L1 expression was higher in well and moderately differentiated carcinomas, there wasn't any statistical significance. In the meta-analysis by Gu *et al.,* too, there was no clear correlation between PD-L1 expression and tumour differentiation.(23) In the studies of Eto *et al.,* and Yamashita *et al.,* the poorly differentiated tumours had an increased PD-L1 expression, however there was no statistical significance for both the studies (24,25).

PD-L1 expression was more evident in higher stages of the tumour i.e., those belonging to stages pT4a and pT4b, as compared to pT2 and pT3 and this correlation was of statistical significance. This is in concordance with several other studies. In the study by Yamashita *et al.,* the incidence of PD-L1 positive tumours was much higher (66.7%) among the cases with stage pT3 and pT4 when compared to cases with pT2 (33.3%).(24) In the study by Kim *et al.,* 94.2% of the cases with a higher depth of invasion (pT2, pT3, and pT4) showed positivity for PD-L1(18). This shows that PD-L1 positivity in gastric cancers has a positive correlation with increase in the depth of invasion.

Tumor node involvement did not show correlation with expression of PD-L1 in our study. Among the resected specimens, there was a greater PD-L1 positivity among the tumours with lymphovascular invasion, however statistically insignificant. In the present study, most of the cases with perineural invasion were PD-L1 negative.

CONCLUSION

The present study has shown that the expression of PD-L1 is seen in a significant number of cases in gastric carcinomas in South India, as in other countries which have similar high rates of this tumour. This becomes an important factor to consider while planning treatment strategies for patients with gastric adenocarcinoma. Thus, it may be stated that routine testing for PD-L1 positivity should form part of the armamentarium of tests done in gastric carcinomas which will help to improve the prognosis by facilitating the addition of targeted antitumour therapy along with conventional treatment modalities. The present study has been done in a small number of

patients and further studies in a larger cohort of patients would be needed to further substantiate these findings.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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