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Original Research Article

Evaluation of PD-L1 Expression on Gastric Cancer and Its Relationship with Clinico-Pathological Variables

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Abstract:

Introduction: Gastric Cancer (GC) is one of the leading causes of cancer related deaths worldwide, necessitating exploration of novel therapeutic avenues. Programmed death-ligand 1 (PD-L1) expression on tumor cells in GC has allowed this to be a promising target for immunotherapy. However, studies evaluating PD-L1 expression in Indian GC patients are limited, prompting our investigation.

Material and Methods: In this prospective observational study, we assessed PD-L1 expression in 40 GC cases using immunohistochemistry (IHC) on tissue samples obtained from small biopsies or gastrectomy specimens. Clinicopathological variables like gender, age, tumor location, tumor size, histopathological diagnosis, grading and staging were recorded. The Combined Positive Score (CPS) was used to evaluate PD-L1 expression, and SPSS software was utilized for statistical analyses.

Results: Our analysis revealed PD-L1 positivity in 29 (72.5%) patients. No significant association was observed with gender, age, tumor location, tumor size, histopathological diagnosis, grading or staging with CPS ≥ 1. Tumor size showed a significant association with PD-L1 expression having CPS ≥ 5. There was also significant association of PD-L1 expression (CPS ≥10) with age and grade of the tumor.

Conclusion: Our study provides insights into PD-L1 expression patterns in GC in the Indian scenario, demonstrating significant association of CPS with tumor size, age and grade of tumor. While limitations such as small sample size and single center design exist, our study demonstrates the potential utility of PD-L1 as a therapeutic target in GC. To confirm these findings and provide guidance for personalized treatment methods in GC patients, additional research with bigger cohorts is necessary.

Keywords: Gastric cancer, Programmed death-ligand 1, Immunohistochemistry, Combined Positive Score (CPS).

Introduction

According to GLOBOCON 2020 estimates, gastric cancer (GC) is the fifth leading cancer overall and third most common cause of cancer related deaths in both sexes combined [1],[2]. According to National Survey of Cancer mortality, in the Indian scenario, GC is the second leading cause of cancer related mortality [3]. The highest incidence in India is noted in the Aizawl district of Mizoram [4]. GC is a heterogenous disease with characteristic phenotype, genotype and clinical profile, including sensitivity to different modalities of treatment and having a varied prognosis. Most sporadic gastric cancers are related to Helicobacter pylori infection. In the developing world, attempts have been made in preventing and treating Helicobacter pylori infections which has impacted the overall incidence rate and epidemiology of GC [5]. Cancer immunotherapy has opened new horizons for GC treatment with astonishing results. PD-1 is expressed on cytotoxic T-cells and other immune cells, while PDL-1 ligand is expressed on normal cells. Normal cells use this PD-1/PDL-1 interaction to inactivate the T-cells, thereby limiting damage to normal tissue. Many tumor cells upregulate PDL-1 expression in order to evade the body's natural immune response. They use the PD-1/PDL-1 signalling just like normal cells to render the T-cells inactive, thereby escaping the immune cycle. The basis for using immunotherapeutic agents is to prevent this PD-1/PDL-1 interaction, thus keeping the immune

system active, preventing immunosuppression. Immune checkpoint inhibitor drugs have shown promising results, especially in recurrent, locally advanced or metastatic GC [6],[7]. At present many studies are ongoing which aim to evaluate the status of PD-L1 expression in GC and correlate it with clinico-pathological parameters. But the number of studies on GC in the Indian scenario is limited. So our primary objective is to evaluate PD-L1 expression in GC in the Indian context and study its relationship with clinico-pathological variables.

Materials and Methods

This was a prospective observational study conducted in the Department of Pathology, IGIMS, Patna, Bihar, over a period of one year from 2022 to 2023, and included 40 diagnosed GC cases in small biopsies or resection specimens. All tissue samples (small biopsies, gastrectomy specimens) from stomach diagnosed histopathologically as GC were included in the study. Patients who received neoadjuvant chemotherapy and those having a history of other primary cancer were excluded from the study.

The small biopsies and resection specimens received in Pathology Department were fixed in 10% formalin solution and grossing was done according to validated protocol. Haematoxylin and eosin stained slides of tissue samples were examined for histopathological variables. Immunohistochemistry (IHC) with PD-L1 was done and its expression including intensity and proportion were evaluated and correlated with the histomorphological findings. After that, Combined positive score (CPS) was calculated.

Specimens included 27 small biopsies and 13 gastrectomy specimens. Classification and grading of tumors were done according to 2016 WHO classification of gastric tumors.

The clinico-pathological parameters studied included the gender, age of patient, tumor location, size, histopathological diagnosis, grade, pTNM staging (according to American Joint Committee on Cancer eighth edition), lymphovascular invasion and perineural invasion. PD-L1 expression was also evaluated in all the cases and correlated with the different clinico-pathological parameters. We took the approval of Institutional Ethics Committee, IGIMS, Patna before conducting this study.

IHC staining and Evaluation: 5µm thin paraffin tissue sections were cut and IHC was performed manually. For the evaluation of PD-L1 expression in GC, we used anti PD-L1 antibody (Clone: IHC 411). A minimum of 100 viable tumor cells were required for optimum assessment of PD-1 expression. For specimens with less than 100 viable tumor cells, tissues from a deeper level of the block or potentially another block helped us achieve a sufficient number of tumor cells for evaluation of PD-L1 expression.

PD-L1 expression in GC was determined by using CPS, which is defined as the number of PD-L1 staining cells (tumor cells, lymphocytes and macrophages) divided by the total number of viable tumor cells, and this result is multiplied by 100. Although the result of the calculation can exceed 100, the maximum score is defined as 100.

We graded the CPS in our study as CPS < 1, CPS ≥ 1, CPS ≥ 5, and CPS ≥ 10.

Statistical Analysis: All analysis was carried out on SPSS 22.0 software. Chi-Square test and Fisher's exact test were used for correlation. P-value < 0.05 was considered as statistically significant.

Results

Out of the 40 patients of GC, males were 23 in number (23/40, 57.5%). 26 patients belonged to age of less than 55 years (26/40, 65%). In 18 patients, tumor was located in the cardia and fundus (18/40, 45 %). 33 patients had tumor size greater than or equal to 3cm (33/40, 82.5%). Histopathological diagnosis of adenocarcinoma was made in 34 patients (34/40, 85%). The predominant grade seen among the patients was moderately differentiated adenocarcinoma (FIG-1), which was noted in 22 patients (22/40, 55%) (Table 1).

None of the clinico-pathological parameters showed significant association with PD-L1 expression of CPS ≥ 1 (Table 2). Tumor size was significantly correlated with CPS of ≥ 5 (Table 3). There was statistically significant association of patient's age and grade of the tumor with CPS ≥ 10 (Table 4).

Table 1: Clinicopathological features of Gastric cancer

Characteristics		Number of patients
Gender	Male	23
	Female	17
Age (years)	<55	26
	≥ 55	14
Samples	Biopsy	27
	Gastrectomy specimens	13
Tumor location	Cardia and fundus	18
	Fundus and body	7
	Pylorus and antrum	15
Tumor size	<3 cm	7

	≥ 3 cm	33
Histopathological diagnosis	Adenocarcinoma	34
	Signet ring carcinoma/others	6
Tumor grade	G1	0
	G2	22
	G3	18
pT stage	T1/T2	6
	T3/T4	7
pN stage (Nodal involvement)	N0	3
	>/=N1	10
Metastasis	Present	0
Lymphovascular invasion	Present	0
Perineural invasion	Present	0

Table 2: Correlation of Clinicopathological parameters with CPS ≥ 1

Characteristics		**CPS < 1**	**CPS ≥ 1**	**p-value**
Gender	Male	8	15	0.23
	Female	3	14	
Age (in years)	<55	6	20	0.39
	≥ 55	5	9	
Tumor location	Cardia and fundus	7	11	0.33
	Fundus and body	1	6	
	Pylorus and antrum	3	12	
Tumor size	<3 cm	2	5	0.94
	≥ 3 cm	9	24	
Histopathological diagnosis	Adenocarcinoma	10	24	0.51
	Signet ring carcinoma/others	1	5	
Tumor Grade	G1	0	0	0.45
	G2	5	17	
	G3	6	12	
pT stage	T1/T2	1	5	0.61
	T3/T4	2	5	
pN stage (Nodal involvement)	N0	1	2	0.63
	≥ N1	2	8	
Metastasis	Present	0	0	--
Lymphovascular invasion	Present	0	0	--
Perineural invasion	Present	0	0	--

Table 3: Correlation of Clinicopathological parameters with CPS ≥ 5

Characteristics		**CPS < 5**	**CPS ≥ 5**	**p-value**
Gender	Male	10	13	0.36
	Female	5	12	
Age (in years)	< 55	8	18	0.23
	≥ 55	7	7	
Tumor location	Cardia and fundus	4	14	0.07
	Fundus and body	2	5	
	Pylorus and antrum	9	6	
Tumor size	< 3 cm	5	2	0.04*
	≥ 3 cm	10	23	
Histopathological diagnosis	Adenocarcinoma	12	22	0.49
	Signet ring carcinoma/others	3	3	
Tumor Grade	G1	0	0	0.22
	G2	8	14	
	G3	10	8	
pT stage	T1/T2	2	4	0.41
	T3/T4	1	6	
pN stage (Nodal involvement)	N0	2	1	0.41
	>/=N1	4	6	

Metastasis	Present	0	0	--
Lymphovascular invasion	Present	0	0	--
Perineural invasion	Present	0	0	--

*Statistically significant

Table 4: Correlation of Clinicopathological parameters with CPS ≥ 10

Characteristics		**CPD < 10**	**CPS ≥ 10**	**p-value**
Gender	Male	15	8	0.25
	Female	8	9	
Age (in years)	< 55	10	16	0.0009*
	≥ 55	13	1	
Tumor Location	Cardia and fundus	10	8	0.70
	Fundus and body	5	2	
	Pylorus and antrum	8	7	
Tumor size	< 3 cm	6	2	0.29
	≥ 3 cm	18	15	
Histological diagnosis	Adenocarcinoma	19	15	0.62
	Signet ring carcinoma/others	4	2	
Tumor Grade	G1	0	0	0.01*
	G2	9	13	
	G3	14	4	
pT stage	T1/T2	5	1	0.90
	T3/T4	6	1	
pN stage (Nodal involvement)	N0	2	1	0.32
	≥ N1	9	1	
Metastasis	Present	0	0	--
Lymphovascular invasion	Present	0	0	--
Perineural invasion	Present	0	0	--

*Statistically significant

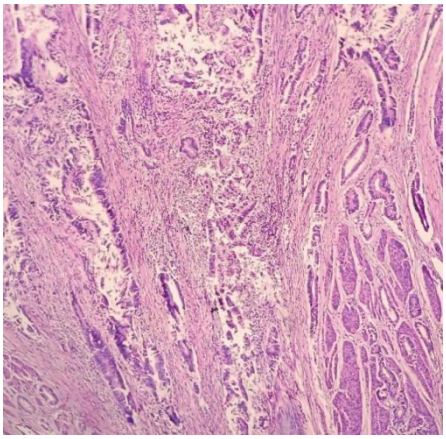

Figure 1: (H & E, 10X) -Moderately differentiated GC.

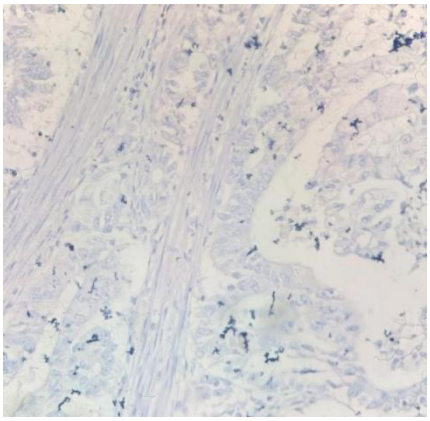

Figure 2: (PDL 1 IHC,40x)-CPS-0

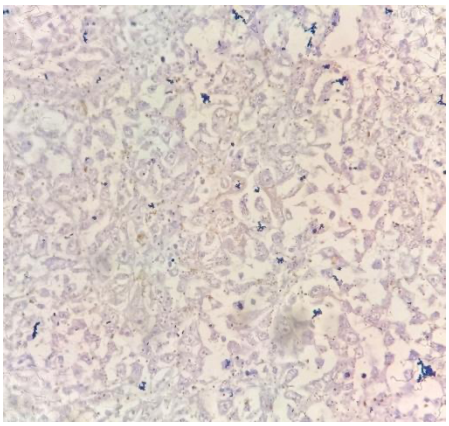

Figure 3: (PDL 1 IHC,40x)-CPS-4

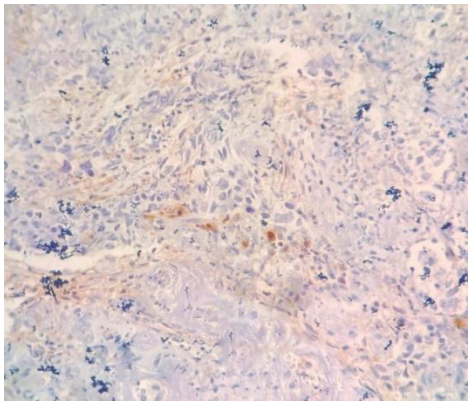

Figure 4: (PDL1 IHC, 40x)-CPS-5

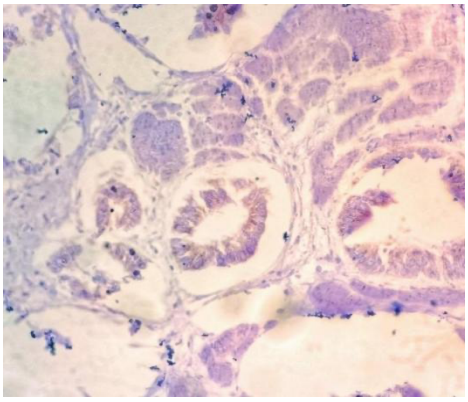

Figure 5: (PDL1 IHC, 40x)-CPS-10

Discussion

PD-1/PD-L1 immune checkpoint inhibitor drugs are used for treating advanced GC patients with CPS ≥ 1[8-9]. The evaluation of PD-L1 expression by IHC is validated for the use of PD-1/PD-L1 inhibitor drugs [10]. The primary objective of our study was to evaluate PD-L1 expression in GC in Indian patients. Out of the total 40 cases, 29 (72.5%) cases showed PD-L1 positivity when cut-off used was CPS ≥ 1(FIG-3). Table 1 showed 27 biopsy cases and 13 gastrectomy cases. The percentage of cases showing PD-L1 positivity was higher than studies from different population (43-63%) [11-15]. Chen et al in a study conducted in 2022 found that 62.3% patients showed positive PD-L1 expression when CPS ≥ 1 was used. They also found that the percentage of patients showing positive PD-L1 expression dropped to 49.2% when CPS ≥ 5 was used. But in our study, we found that 62.5% patients showed PD-L1 positivity with CPS ≥ 5. Eventually when CPS ≥/10 was used as cut-off, 42.5% patients showed PD-L1 positivity, which was higher than the study by Chen et al [16]. Chinese researchers studied 550 patients and came up with result of 37.3% positivity [17]. Asian researchers demonstrated 22.8% PDL-1 expression [18]. In the CheckMate-032 trial, the percentage of patients having PD-L1 expression with CPS ≥ 1, ≥ 5 and ≥ 10 were 32%, 10% and 8% respectively.

Dung et al studied 54 patients and found that PD-L1 positivity (CPS ≥ 1) showed significant correlation with tumor location [19]. This contrasted with the study of Saito et al [20]. On the other hand, our study showed a significant correlation of PD-L1 expression with tumor size (p-value 0.04) when CPS ≥ 5(FIG -4) was used. PD-L 1expression exhibited significant statistical correlation with depth of invasion [21], lymph node metastasis [22-23]and vascular invasion [24].

Interestingly, when CPS ≥ 10 (FIG-5) was used we found significant correlation of PD-L1 with age of patient and tumor grade. This is contradictory to the findings of Eto et al [15]. There was no association of PD-L1 expression (CPS ≥ 1) with parameters like age, gender, tumor location, tumor size, histopathological diagnosis, grading, pT and pN stage. USA Food and Drug Administration has approved Pembrolizumab as an immune checkpoint inhibitor drug in GC patients with positive PD-L1 expression.

Varied factors like patient cohort, ethnicity, different types of specimens, IHC staining method and different cut-off levels used for PDL-1 evaluation may be responsible for the difference in expression rate of PD-L1. We used PD-L1 IHC Clone 411 to evaluate the correlation between PD-L1 expression and various clinico-pathological parameters. On the other hand, KEYTRUDA is a humanized monoclonal PD-1-blocking antibody. Above mentioned companion diagnostic assay was approved by the FDA, at the CPS level ≥ 1 to assess PD-L1 expression in GC [25]. Researchers have also indicated that GC patients had a significantly better overall survival with CPS <1(FIG-1) [26]. On the other hand, Schoemig-Markiefka et al concluded that there is increased responsiveness to immune check point inhibitors drugs whose CPS was greater than or equal to 1[27].

Limitations

Manual method of IHC staining, type of clone used, varied cut-off for PD-L1 reactivity and small sample size were the responsible factors for discordant results.

Conclusion

There is a huge variation of PD-L1 expression in different studies worldwide. In India, this study is going to be the first study highlighting few facts which are aberrations from western world findings. First finding indicates 72.5% GC patients showing PDL-1 expression (CPS ≥ 1). Second finding is that PD-L1 positivity with CPS cut-off ≥ 5 has a statistically significant correlation with tumor size, CPS cut-off ≥ 10 has a significant correlation with age of patient and grade of tumor. Large scale multicentric studies should however be conducted to validate these findings.

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