RESEARCH ARTICLE

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Expression of Programmed Death Ligand1 (PD-L1) in Gastric Carcinoma (Histopathological and Immunohistochemical Study)

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Abstract

Objectives: To evaluate immunohistochemical expression of PD-L1 in cases of gastric adenocarcinoma. To correlate PD-L1 immuno-histochemical expression with other available clinico-pathological parameters such as age, sex, grade, stage, lymph node (L.N) metastasis and others. **Material and methods:** The present retrospective study retrieved the data and archived paraffin blocks of 60 cases of Gastric carcinoma. Immunohistochemical evaluation was done to assess the expressions of PD-L1 in the tumor cells (TC), tumor infiltrated lymphocytes (TILs) and combined positive score (CPS). **Results:** TC PD-L1 expression was detected in 56.7% of cases, TILs PD-L1 expression was detected in 53.3 % of cases and CPS PD-L1 expression was detected in 63.3% of case, with no statistically significant correlation with clinico-pathological parameters except TILs PD-L1 expression showed statistically significant correlation with positive TILs (P value <0.019). **Conclusion:** Our findings supported the expression of PD-L1 by TC, TILs, and CPS in gastric cancer, with increased expression in a subpopulation of TILs rich in PD-L1 identifying them as potential targets for PD-1/PD-L1 therapy.

Keywords: Gastric cancer -PD-L1 - Tumor cells –TILs-CPS

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Introduction

Gastric cancer (GC) is a serious health problem, being the 5th most common cancer and the 3rd worldwide leading cause of cancer death, with an estimated one million newly diagnosed cases and 783,000 patients died in 2018 (Chen et al., 2019). Common malignancies include GC, which is most prevalent in Eastern Asia (Korea, Mongolia, Japan, and China), Central and Eastern Europe, and South America. Northern America and Africa have the lowest death rates from GC (Gu et al., 2017).

Gastric carcinoma is the 12th most prevalent cancer in Egypt, affecting both sexes equally. With 1271 new cases in 2015, it accounts for 1.6 percent of all diagnosed malignancies and 2.2 percent of all cancer deaths in Egypt (Darwish et al., 2016). The prognosis of G.C is poor, this is mostly due to absence of symptoms in early stages, therefore delayed diagnosis and treatment (Wu et al., 2017). The bacterium helicobacter pylori is well recognized cause of gastric cancer, in particular non-cardiac cancer. Epstein-Barr virus (EBV), which is known to be carcinogenic, has also been linked to gastric cancer in some studies. Smoking, alcoholic consumption, obesity and foods preserved by salting are probably risk factors for gastric cancer (Ferlay et al., 2012).

One important immune response checkpoint that is addressed by cancer immunotherapy is the programmed death (P D) 1 pathway (Cimino-Mathews et al., 2015). PD-1 is temporarily expressed on the surface of activated T-cells and other immune cells including natural killer (NK) T-cells, B-cells, activated monocytes and dendritic cells (Tumeh et al., 2014).When PD-1 binds to its ligand PD-L1, which is expressed constitutively on many different cell types, the PD-1-expressing cell undergoes apoptosis. Consequently, the immunological response can be effectively limited (Pardoll, 2012).

PD-1 and PD-L1 interaction can limit T-cell activity and act as a T-cell co-inhibitory signals. This is important to maintain peripheral tolerance and prevent massive tissue damage during clearing of the infection. However, in the tumor microenvironment, PD-L1 is up regulated on the tumor cells, resulting in defect in the anti-tumor immune response an escape of malignant cells from detection by cytotoxic T-cell (Silva et al., 2016). Immunotherapy has gained popularity in the world of cancer treatment. The "Breakthrough of the Year" award for cancer immunotherapy was given in large part because of the promising outcomes of the immune-checkpoint blockage

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therapy (Couzin-Frankel., 2013).

The inhibition of programmed death-1 (PD-1) and programmed death-ligand 1 is one of the most often used mechanisms underlying immunotherapy (PD-L1). Tumor immune evasion is caused by the PD-1/PD-L1 interaction, which functions as an inhibitory factor in the final stage of the cancer immunity cycle. It causes functional impairment of antigen-specific T cells (Pardoll, 2012) and (Chen and Mellman, 2013).

Materials and Methods

Study Design and Patients'data

The study included 60 cases of gastric carcinoma that underwent total or subtotal gastrectomy. The study's data were gathered as formalin-fixed, paraffin-embedded tissue blocks between January 2016 and September 2019 from the pathology department archives at Kasr AL-Ainy, the Faculty of medicine at Cairo University, and a private laboratory. Cases were included if they have confirmed diagnosis of gastric carcinoma. We excluded cases who had undergone prior preoperative radiation, chemotherapy or targeted therapy, and Cases with evidence of other types of malignant neoplasms or having an associated benign gastric lesion. The following data were collected from the records of eligible cases before our histopathological reevaluation and immunohistological staining: sex, age at presentation, site of tumor, histologic diagnosis of the tumor, World Health Organization (WHO) grading.

Histopathological Examination

The paraffin blocks were sectioned at 3-4 μm thick and stained with routine Hematoxylin and Eosin stain. The grading of the tumor and histopathological classification were done in concordance with the WHO classification of digestive system ,

PDL-I Immunohistochemical Staining and evaluation

Immunostaining was done using BenchMark XT (Ventana) autostainer. Human tonsil sections were used as a positive control for cases stained for PDL-1 because they show significant staining in some crypt epithelial regions and weak to moderate staining of the follicular macrophages in the germinal centres. The surface epithelium, fibroblasts, and endothelium should all express PD-L1, which is employed as an internal & negative control ("Interpretation Manual - Gastric," 2019). Staining intensities and percentages were scored in both the tumor cells (TC) and the TILs.

Scoring of PD-L1 in Tumor cells

For PD-L1 staining of tumor cells, membrane staining alone was taken as positive and further subjected to the following immunoreactivity scoring system (IRS) (Park et al.,2014).

A: percentage of stained cells-0 (negative), 1 (≤1% positive), 2 (2%-10% positive), 3 (11%- 50% positive), and 4 (>50% positive cells).

B: staining intensity-0 (no immunostaining), 1 (weak staining/light yellow), 2 (moderate staining/brown), and 3 (strong staining/dark brown). The addition of category A and B resulted in a total score ranging from 0 and 7. A total score of ≤2 was considered negative and >2 was considered positive (Wei et al., 2018).

Scoring of PD-L1 in Tumor infiltrating lymphocytes

For PD-L1 staining of immune cells, membrane or/ cytoplasmic staining was taken as positive and further subjected to the following immunoreactivity scoring system.

• Scoring criteria were: 0 (negative), 1 (1%-5% positive), 2 (6%- 20% positive), or 3 (>20% positive) points, score 0 was considered (negative) while scores 1,2 and 3 were considered (positive) (Wei et al., 2018).

Combined Positive Score PD-L1 expression

PD-L1 staining cells include tumor cells, lymphocytes, and macrophages. The combined positive score (CPS) is calculated by multiplying of PD-L1 staining cells by the total number of viable tumor cells (Liu et al., 2020). CPS considered negative if <1, and considered positive if>1. CPS is defined accordingly:

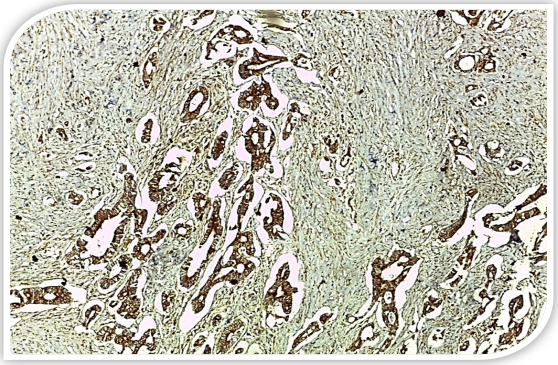
$$\text{CPS} = \frac{\text{\# PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# viable tumor cells}} \times 100$$

Statistical Analysis

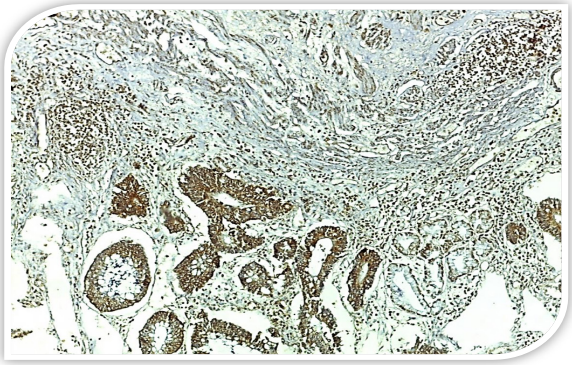
The histopathological and immunohistochemical data were then transferred to the SPSS Software program, version 24 to be statistically analysed. Simple descriptive statistics (arithmetic mean and standard deviation) were used for summary of quantitative data and frequencies were used for qualitative data. Estimation of the association between categorical variables was performed using the chi-square test. P value < 0.05 is considered as statistically significant.

Results

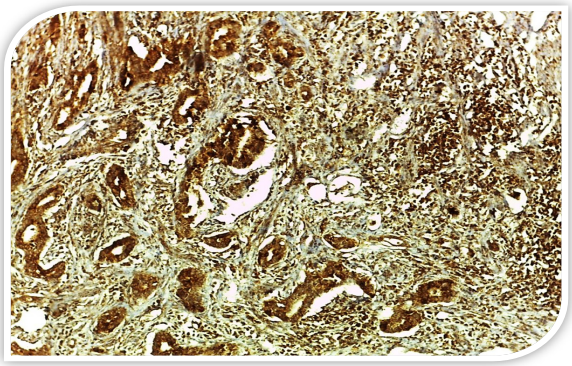
study including 60 cases of gastric carcinoma. Most cases ≤55 years (26 cases) representing 52%. The mean age of cases was 55.56 years, with standard deviation12.477. Range (25-80); median 55 years (data of 10 cases regarding the age could not be identified in the records). Thirty Two cases (53.3%) were males and twenty eight cases (46.7%) were females, with male to female ratio was 1:0.9. Regarding the histological type, most cases were tubular moderately differentiated adenocarcinoma (22 cases, 36.7%). Regarding the histological grade: Among the collected cases, 37 cases (61.7%) were high grade and 23 cases (38.3%) were low grade . Regarding the T stage, most cases were T3 (46.6%), Regarding the lymph nodal metastasis of the studied cases, 10 cases were negative for lymph node metastasis (N0; 16.7%), while 50 cases (83.3%) were positive for metastasis. Among positive cases; 9 cases were N1 (15%), 16 cases were N2 (26.6%) and 25 cases were N3 (41.7%). Regarding tumor size; ≥5 cm were representing most cases (71.7%) .Regarding tumor site; 37 cases were present in the body of the stomach representing 68.5% .out of 54 cases of the total 60 studied cases (N.B. specific site of six cases could



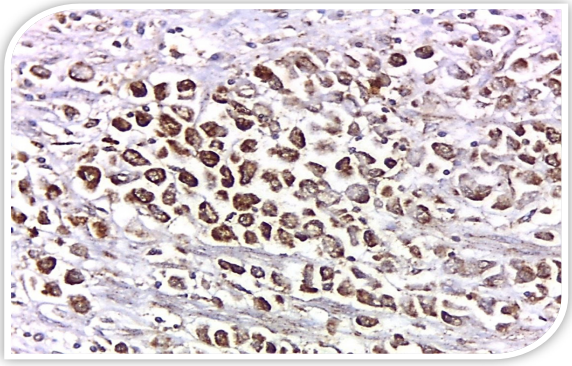
Gastric adenocarcinoma grade 2, showing positive strong membranous PD-L1 expression in tumor cells and TILs). (IHC X400 Original Magnification)



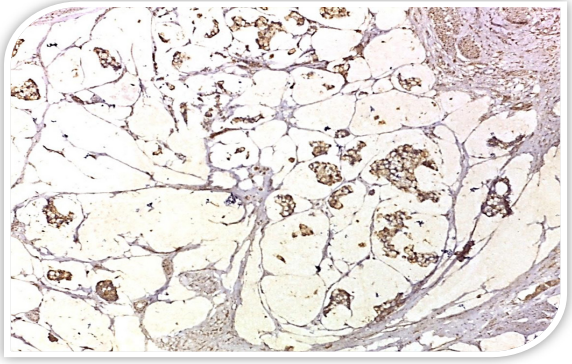
Gastric adenocarcinoma grade 2, showing positive strong membranous PD-L1 expression in both tumor cells and TILs. (H&E X200 Original Magnification)



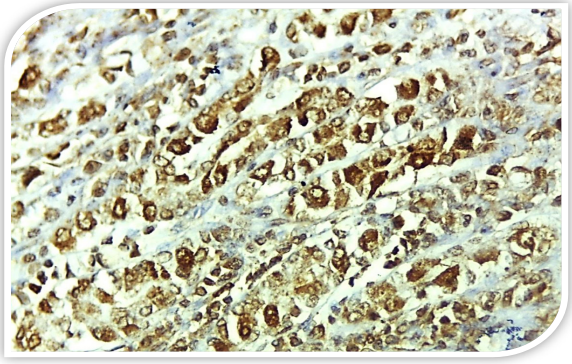
Gastric adenocarcinoma grade 3, showing positive strong membranous PD-L1 expression in both tumor cells and TILs. (H&E X400 Original Magnification)



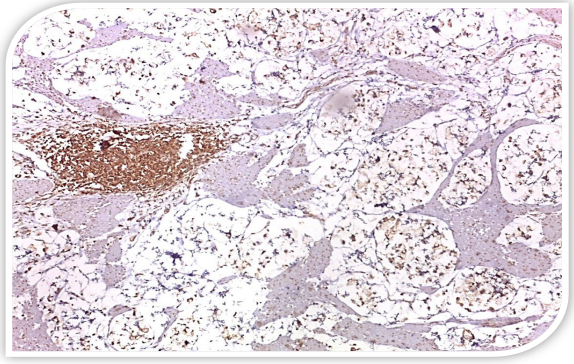
signet ring carcinoma, showing strong positive membranous PD-L1expression. (IHC X400 Original Magnification)



Mucinous adenocarcinoma, showing strong positive membranous PD-L1 expression. (IHC X200 Original Magnification)

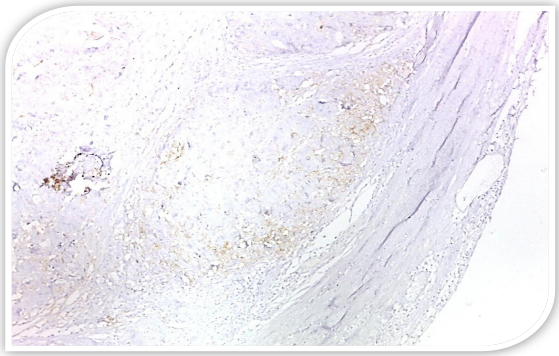


Gastric undifferentiated adenocarcinoma, showing positive membranous PD-L1 expression. (IHCX400 Original Magnification)

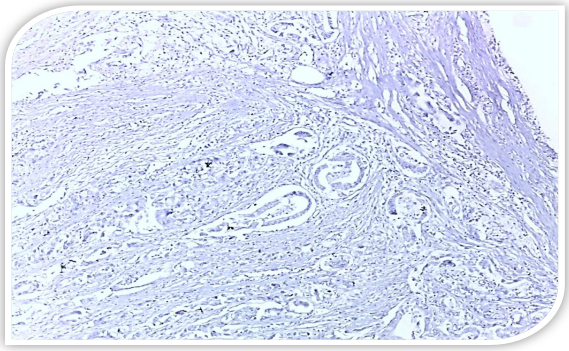


Gastric adenocarcinoma, showing moderate intensity of PD-L1 expression. (IHC X200 Original Magnification)

Figure 1. PD-L1 Immunohistochemical Expression



Gastric adenocarcinoma, showing weak intensity of PD-L1 expression. (IHC X100 Original Magnification)



Gastric adenocarcinoma, showing negative of PD-L1 expression. (IHC X100 Original Magnification)

Figure 1. PD-L1 Immunohistochemical Expression

Table 1. The Pathological Characteristics of the Studied Cases. Expression of PD-L1 in gastric carcinoma (Histopathological and immunohistochemical study).

	No	%
Age		
≤55	26/50	52
>55	24/50	48
Gender		
Male	32/60	53.3
Female	28/60	46.7
Diagnosis (histological type)		
Tubular moderately differentiated adenocarcinoma	36.7	22
Tubular poorly differentiated adenocarcinoma	20	12
Signet ring adenocarcinoma	23.3	14
Mucinous adenocarcinoma	6.7	4
Undiffrentiated adenocarcinoma	13.3	8
Grade		
Low grade	38.3	23
High grade	61.7	37
T stage (depth of invasion)		
T2	16.7	10
T3	46.6	28
T4	36.7	22
N stage		
N0	16.7	10
N1	15	9
N2	26.6	16
N3	41.7	25
Node metastasis		
Absent	16.7	10
Present	83.3	50
Total	100	60
Tumor size		
<5	28.3	17/60
≥5	71.7	43/60
Tumor site		
Body	68.5	37/54
Pylorus	31.5	17/54

Table 1. Continued

	No	%
Lymphovascular invasion (LVI)		
Absent	8.3	5 / 60
Present	91.7	55/60
Perineural invasion (PNI)		
Absent	43.3	26/60
Present	56.7	34/60
Tumor infiltrated lymphocyte (TILs)		
Negative	51.7	31/60
Positive	48.3	29/60

not be identified from the records) .Regarding the evidence of Lympho-vascular invasion (LVI), 55 cases were found to show LVI (91.7%), Regarding the peri-neural invasion (PNI), 34 cases were found to show invasion (56.7%). Regarding the extent of TILs, 31 cases showed negative TILs (51.7%) and 29 cases showed positive TILs (48.3%). The pathological characteristics of the studied cases are summarized in (Table 1).

PD-L1 Expression

In the current study, total score of ≤2 was considered negative and >2 was considered positive. Thirty four 34 cases (56.7%) showed positive PD-L1 expression in tumor cells (TC). Among the positive cases, 5 cases (8.3%) were score 3, 3 cases (5%) were score 4, 3 cases (5%) were score 5, 4 cases (6.7%) were score 6 and 19 cases (31.7%) were score 7. Regarding PD-L1 immunohistochemical expression in TILs, score 0 was considered (negative) while scores 1, 2 and 3 were considered (positive). PD-L1 immunohistochemical expression in TILs was positive in 32 cases (53.3%) . Among the positive cases, 8 cases (13.3%) were score 1, 5 cases (8.3%) were score 2 and 19 cases (31.7%) were score 3. CPS PD-L1 immunohistochemical expression was positive in 38 cases (63.3%).

Regarding the relation between the age and gender of the studied cases on one side and Tumor cell(TC) PD-L1 expression on the other side, cases of both age groups showed 50%positive PD-L1expression (P value = 0.768, insignificant) . Females showed more expressionrate52.9%

Table 2. PD-L1 Expression Score Expression of PD-L1 in Gastric Carcinoma (Histopathological and immunohistochemical study)

	No	%
TC PD-LI score		
Score 0	24	40
Score 2	2	3.3
Score 3	5	8.3
Score 4	3	5
Score 5	3	5
Score 6	4	6.7
Score 7	19	31.7
TC PD-L1		
Negative	26	43.3
Positive	34	56.7
TIL PD-LI Score		
Score 0	28	46.7
Score 1	8	13.3
Score 2	5	8.3
Score 3	19	31.7
TIL PD-L1		
Negative	28	46.7
Positive	32	53.3
CPS PD-L1		
Negative	22	36.7
Positive	38	63.3

(P value = 0.265, insignificant). PDL-1 scoring illustrated in (Table2).

Regarding the relation between the age and gender of the studied cases and TILs PD- L1, age group >55 years showed (52%) higher positive PD-L1 expression and showed no difference in positive PD-L1 expression between males and females (P value = 0.571 and 0.580 respectively, insignificant) .

Regarding the relation between the age & gender of the studied cases and CPS PD-L1 expression, age group ≤55years showed higher positive PD-L1 expression (53.3%) ,and females showed more expression rate 52.6% (P value = 0.817 and 0.224 respectively, insignificant).

Regarding the relation between the various histological types and TC, TILs and CPS PD-L1 expression. Tubular moderately differentiated adenocarcinoma showed the highest rate of PD-L1 expression (32.4 %, P value = 0.273, insignificant), (31.2 %, P value = 0.282, insignificant), and (31.6%, P value = 0.408, insignificant) respectively .

On studying the relation between tumor grade and TC, TILs and CPS PD-L1 expression, high grade cases showed higher rate of expression (67.6%, P value = 0.276, insignificant), (68.8%, P value = 0.228, insignificant), and (68.4%), P value = 0.157, insignificant) respectively.

As for tumor (T) pathological stage, cases classified as T3 showed the highest TC , TILs and CPS PD-L1 expression rate (41.2%, P value = 0.251, insignificant), (46.9%, P value = 0.435, insignificant) and (42.1%, P value = 0.436, insignificant) respectively.

As for the lymph node metastasis cases with positive metastasis showed highest TC, TILs and CPS PD-L1 expression (82.4%, P value =0.816, insignificant), (84.4%, P value =0.817, insignificant) and (84.2%, P value =0.811, insignificant) respectively .

Among the N stage, a high rate of positivity for PD-L1 was observed with the increase in the N stage. Where TC PD-L1 was positive in 17.6% N0 and N1 cases, 23.5% N2 cases, and 41.2% N3 cases. (P value = 0.872, insignificant), TILs PD-L1 was positive in 15.6% N0 and N1 cases, 21.9% N2 cases, and 46.9% N3 cases.(P value = 0.777, insignificant), and CPS PD-L1 was positive in 15.8% N0 and N1 cases, 21.1% N2 cases, and 47.3% N3 cases. (P value = 0.547, insignificant) .

Regarding the relation between tumor size and TC, TILs and CPS PD-L1 expression, cases with tumor size ≥5 cm showed the highest rate of PD-L1 expression (70.6%, P value = 0.832, insignificant), (75%, P value = 0.540, insignificant) and (73.7% , P value = 0.649, insignificant) respectively.

Regarding the relation between tumor site and TC,TILs and CPS PD-L1 expression, cases present in body region showed the highest rate of PD-L1 expression (73.3%, P value = 0.394, insignificant), (75.9%, P value = 0.211, insignificant) and (70.6%, P value = 0.669, insignificant) respectively .

As for lympho-vascular invasion(LVI) by the tumor, cases with positive LVI showed highest rate of TC, TILs and CPS PD-L1 expression (88.2%, P value = 0.377, insignificant), (93.8%, P value = 0.657, insignificant) and (89.5%, P value = 0.643, insignificant) respectively.

As for perineural invasion (P.N.I) by the tumor, cases with positive invasion showed highest rate of TC, TILs and CPS PD-L1 expression (52.9%, P value = 0.505,insignificant),(56.3%, (P value = 0.944, insignificant) and (57.9%, P value = 0.801, insignificant) respectively.

In this study, cases with positive TILs showed highest rate of TC, TILs and CPS PD-L1 expression (52.9%, P value = 0.414, insignificant), (62.5%, P value = 0.019, significant) and (52.6%, P value = 0.381, insignificant) respectively. The pathological characteristics of the PDL-1 expression in studied cases are summarized in (Table 3) /Figure 1.

Discussion

Gastric carcinoma is one of the most common and fatal malignancy worldwide. The prognosis of gastric carcinoma remains poor despite the use of multidisciplinary treatments. So there was an urgent need to develop new therapeutic modalities in gastric carcinoma such as immunotherapy and targeted therapy. And Anti-PD-L1 is one of the; recently described therapies. PDL-1 abolishes the antitumor immune response through induction of apoptosis of cytotoxic T cells (Soliman and Ibrahim, (2020). In this study, we recruited 60 tumor sections from Gastrectomy specimens collected from the pathology department at the Kasr el Aini hospital and some private laboratories in the period between January 2016 and September 2019.

Table 3. PD-L1 Expression of the Studied Cases are Summarized. Expression of PD-L1 In gastric carcinoma(Histopathological and immunohistochemical study)

	TC PD-L1					TILs PD-L1					CPS PD-L1				
	Negative No=26		Positive No=34		P	Negative No=26		Positive No=34		P	Negative No=26		Positive No=34		P
	No	%	No	%		No	%	No	%		No	%	No	%	
Age (total 50 cases)															
≤55	13/24	54.2	13/26	50	0.768 NS	14/25	56	45992	48	0.571 NS	44105	50	16/30	53.3	0.817 NS
>55	45597	45.8	13/26	50		45962	44	13/25	52		44105	50	14/30	46.7	
Sex (total 60 cases)															
Male	16/26	61.5	16/34	47.1	0.265	16/28	57.1	16/32	50	0.58 NS	14/22	63.6	18/38	47.4	0.224 NS
Female	46296	38.5	18/34	52.9	NS	47088	42.9	16/32	50		44774	36.4	20/38	52.6	
Histological type`															
Tubular moderately differentiated adenocarcinoma	11	42.3	11	32.4	0.273 NS	12	42.9	10	31.2	0.282 NS	10	45.5	12	31.6	0.408 NS
Tubular poorly differentiated adenocarcinoma	4	15.4	8	23.5		5	17.9	7	21.9		3	13.6	9	23.6	
Signet ring carcinoma	8	30.8	6	17.6		8	28.5	6	18.7		6	27.3	8	21.1	
Mucinous adenocarcinoma	2	7.7	2	5.9		2	7.1	2	6.3		2	9.1	2	5.3	
Undiffrentiated carcinoma	1	3.8	7	20.6		1	3.6	7	21.9		1	4.5	7	18.4	
Grade															
Low grade	12	46.2	11	32.4	0.276 NS	13	46.4	10	31.2	0.228 NS	11	50	12	31.6	0.157 NS
High grade	14	53.8	23	67.6		15	53.6	22	68.8		11	50	26	68.4	
T Stage															
T2	2	7.7	8	23.5	0.251 NS	3	10.7	7	21.9	0.435 NS	2	9.1	8	21.1	0.436 NS
T3	14	53.8	14	41.2		13	46.4	15	46.9		12	54.5	16	42.1	
T4	10	38.5	12	35.3		12	42.9	10	31.2		8	36.4	14	36.8	
L.N															
Abscent	4	15.4	6	17.6	0.816 NS	5	17.9	5	15.6	0.817 NS	4	18.2	6	15.8	0.811
Present	22	84.6	28	82.4		23	82.1	27	84.4		18	81.8	32	84.2	NS
N stage															
N0	4	15.4	6	17.64	0.872 NS	5	17.9	5	15.6	0.777 NS	4	18.2	6	15.8	0.547 NS
N1	3	11.5	6	17.64		4	14.3	5	15.6		3	13.6	6	15.8	
N2	8	30.8	8	23.52		9	32.1	7	21.9		8	36.4	8	21.1	
N3	11	42.3	14	41.2		10	35.7	15	46.9		7	31.8	18	47.3	
Tumor size															
<5	7	26.9	10	29.4	0.832 NS	9	32.1	8	25	0.54 NS	7	31.8	10	26.3	0.649 NS
≥5	19	73.1	24	70.6		19	67.9	24	75		15	68.2	28	73.7	

Table 3. Continued

	TC PD-L1					TILs PD-L1					CPS PD-L1				
	Negative No=26		Positive No=34		P	Negative No=26		Positive No=34		P	Negative No=26		Positive No=34		P
	No	%	No	%		No	%	No	%		No	%	No	%	
Tumor site															
Body	15	62.5	22	73.3	0.394 NS	15	60	22	75.9	0.211 NS	13	65	24	70.6	0.669 NS
Pylorus	9	37.5	8	26.7		10	40	7	24.1		7	35	10	29.4	
LVI															
Absent	1	3.8	4	11.8	0.377 NS	3	10.7	2	6.2	0.657 NS	1	4.5	4	10.5	0.643 NS
Present	25	96.2	30	88.2		25	89.3	30	93.8		21	95.5	34	89.5	
PNI															
Absent	10	38.5	16	47.1	0.505 NS	12	42.9	14	43.7	0.944 NS	10	45.5	16	42.1	0.801 NS
Present	16	61.5	18	52.9		16	57.1	18	56.3		12	54.5	22	57.9	
TILS															
Negative	15	57.7	16	47.1	0.414 NS	19	67.9	12	37.5	0.019 NS	13	59.1	18	47.4	0.381 NS
Positive	11	42.3	18	52.9		9	32.1	20	62.5		9	40.9	20	52.6	

In this study, positive expression of PDL-1 was found in tumor cell (TC) in 34 cases (56.7%) of the cases, in tumor infiltrating lymphocytes (TILs) in 32 cases (53.3%) and in combined positive score (CPS) in 38 cases (63.3%). Many studies reported that PDL-1 overexpression in gastric carcinoma ranges from 15 to 75% of patients (Wang et al., (2018). On the contrary, Soliman and Ibrahim, (2020) observed that positive expression of PDL-1in TC, TILs, and CPS was found in 14%, 27% and 28% of cases respectively, Also Wang et al., (2018) observed that its expression in tumor cells was 17.3 and 34.5% in immune cells. Xing et al., (2018) show lower PDL-1 expression in tumor cells (37.8%) but higher PDL-1 expression in immune cells (74.8%) than ours.

The various antibodies utilised in each investigation, as well as the various methodologies (manual vs. automated, entire tumour blocks, or endoscopic biopsy) and interpretations, can be used to explain the significant differences in the expression pattern of PDL-1 (different cutoff value of positivity).

Regarding the age of patient, in our study age ≤55 showed the highest rates of PD-L1 expression in TC,TIL and CPS and CPS, but there was no significant association between them. This agreed with the results of other studies that showed high rate of PD-L1 expression in age group <59 years with no significant correlation between them Fang et al., (2017) and Soliman and Ibrahim,(2020). On the other hand, Wang et al., (2018) showed highest rate of PD-L1 expression in old age >60 with significant statistical correlation between them.

Regarding the gender of patient, in our study female gender showed higher rate of PD-L1 expression in TC, TILs and CPS than male. But there was no significant association between them.This agree with results of studies done by Wang et al., (2018) and Rhee et al., (2020) with insignificant correlation, But Soliman and Ibrahim, (2020) showed significant correlation between TILs and CPS PD-L1 expression with gender.

In the present study, regarding the histological subtypes, tubular moderately differentiated and tubular poorly differentiated adenocarcinoma (intestinal type) showed the highest rates of PD-L1 expression in TC, TILs and CPS with insignificant correlation between them. This was similar to the results of Wang et al., (2018) and Soliman and Ibrahim, (2020) which showed that the highest rates of PD-L1 expression was in intestinal type, with significant correlation.

In our study, high grade cases showed higher rates of PD-L1 expression in TC, TILs and CPS than low grade cases, with insignificant correlation. This was consistent with most of the studies did by Ju et al., (2017); Fang et al., (2017); Xing et al., (2018), and Soliman and Ibrahim, (2020). In the other hand Rhee et al., (2020)showed significant correlation between them.

Concerning the T stage, our study showed that T3 exhibited the highest rate of PD-L1 expression on TC, TILs and CPS, with insignificant correlation. Wang et al., (2018 reported similar results. Though, Soliman and Ibrahim, (2020) showed significant correlation.

In this study, cases positive for LN metastasis showed higher rates of PD-L1 expression in TC, TILs and CPS

than LN negative cases, with insignificant correlation. This agreed with study of Wang et al., (2018).

Among the LN positive cases in our study, the rate of PD-L1 expression increased with the increase in the N stage; e.g. PD-L1 expression in TC N1 (17.6%), N2 (23.5%) and N3 (41.2%). This was consistent with the results of Rhee et al., (2020). In the present study, cases with tumor size ≥5 cm showed higher rates of PD-L1 expression on TC, TILs and CPS than tumor size <5 cm, with insignificant correlation between them. This disagree with studies of Wang et al., (2018) and Soliman and Ibrahim (2020).

In the present study, cases with tumor located in body region showed higher rates of PD-L1 expression on TC, TILs and CPS than tumor located in pyloric region, with insignificant correlation between them. This was consistent with the results of Wang et al., (2018). Although, Soliman and Ibrahim (2020) showed high expression in antrum region. In this study, cases positive for LV and peineural invasion showed higher rates of PD-L1 expression on TC, TILs and CPS than LV and peineural negative invasion cases, with insignificant correlation between them. This disagreed with study done by Soliman and Ibrahim, (2020) Which showed high expression of PD-L1 in cases with negative LV and perineural invasion.

In our study, cases with positive TILs (≥ 25%) showed higher rate of PD-L1 expression in TC, TILs and CPS than cases with negative TILs (<25%). Additionally, there was significant correlation between TILs with PD-L1 expression in TILs (P value = 0.019).This was consistent with the results of Ju et al., (2017) and Fang et al., (2017). This result led us to speculate that PD-L1 expression on TILs, rather than tumor cells, could be more relevant to the inhibitory effects of anti PD-L1therapy, and support the use of PD-L1 expression on TILs as a predictive biomarker in GC for immunotherapies.

The different sample size, different variants, different antibodies used to detect PD-L1, absence of universal standard for PD-L1 expression and different methodology enrolled in the studies might explain the contradictory results regarding correlation between PDL-1 expression in gastric carcinoma and other clinico-pathological parameters. Further studies are needed to reveal the precise mechanism of PD-L1 up-regulation in GC.

Author Contribution Statement

All authors contributed efficiently to the research and approved the manuscript..

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Approval

This research was approved by the research committee of pathology department, faculty of medicine, Cairo university and Kasr alainy research ethics committee (REC).

Ethical Declaration

This study obtained the approval of REC that conduct according to appropriate local and institutional regulation.

Data Availability

All data is available upon request according to insititutional regulation and with official permission.

Study registrartion

This study isn't registered in any database (clinical trial, guidelines or meta-analysis).

Conflict of interest

The authors declare that they have no conflict of interest.

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