Research article

A study of immunohistochemical expression of PD-L1 in gastric carcinomaBodepudi Srija¹, Susruthan Muralitharan², Barathi Gunabooshanam³¹Postgraduate, ²Associate Professor, ³Assistant Professor, Department of Pathology, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, Tamil Nadu, India

(Received: June 2021 Revised: July 2021 Accepted: August 2021)

Corresponding author: **Susruthan Muralitharan**. Email: susruthansak@gmail.com**ABSTRACT**

Introduction and Aim: Aberrant over expression of PD L1 by tumours and tumour infiltrating lymphocytes provide an immune shield to the tumours. The present study aims to evaluate the effect of over expression of PD L1 in tumour cells and tumour infiltrating lymphocytes on various clinicopathological aspects of gastric carcinoma including the follow up and survival analysis.

Materials and Methods: Paraffin blocks were retrieved from 100 cases of primary gastric carcinoma who underwent curative resection. Immunostaining was done using a qualitative immunohistochemical assay from Ventana Roche with rabbit monoclonal anti PD-L1 clone SP142 intended for use in the assessment of the PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) tissue.

Results: Out of 100 cases 65 were males and 35 were females. PD-L1 expression is observed in tumour cells in 17 cases and tumour infiltrating immune cells in 44 cases. Statistically significant correlation of expression of PD-L1 was observed with the clinicopathological characteristics like, larger tumour size (p value 0.03), lymphovascular invasion (p value 0.01), lymph node metastasis (p value 0.01) and higher tumour stage (p value 0.02). Two years follow up did not show any statistically significant correlation between PD-L1 expression in tumour cells and tumour infiltrating immune cells and survival (p value 0.27).

Conclusion: Present study shows a substantial expression of PD-L1 in patients with gastric carcinoma. Hence PD-L1 immunohistochemistry can be potentially helpful in screening candidates for anti PD-L1 therapy.

Keywords: PD L1; Gastric cancer; immunohistochemistry; anti PD-L1 therapy

INTRODUCTION

Gastric carcinoma is one of the leading cancers across the world with an approximate million new cases being reported per year (1). Despite advances in diagnosis and treatment the five year survival is only 20%. Further it accounts for nearly 15% of cancer related deaths across the globe. Gastric carcinoma is a malignant epithelial neoplasm which represents a biologically and genetically heterogeneous group of neoplasms with multifactorial aetiologies (2). The etiological factors include infectious agents like *Helicobacter pylori*, dietary factors, lifestyle and occupational changes and genetic factors. The presenting symptoms of gastric cancer are vague and usually present with the triad of anaemia, weight loss and loss of appetite which leads to delayed diagnosis and presentation in advanced stage of the disease (3). Due to this late presentation the only option available will be chemotherapy in most cases. Conventional chemotherapy was not successful in treating these patients with a mean survival of only 10 months. So search for alternate modes of treatment resulted in development of treatment protocols targeting biological markers like Human Epidermal growth factor receptor (HER2) and Mesenchymal epithelial transition factor (c-MET), and immunological strategies i.e. inhibition of immune check points like Programmed death-ligand 1 (PD-L1)

and Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (4- 6). Early reports using these strategies showed promising results but further studies are needed to get better insights into the mechanisms of action of these pathways to refine the treatment protocols.

PD-L1 (also known as CD 274 and B-H7) is a transmembrane ligand for hepatocyte growth factor, acts as an immune check point mechanism to control inflammation and prevent autoimmune reactions. However aberrant over expression of PD-L1 by tumours and tumour infiltrating lymphocytes, prevents cytotoxic T cell mediated anti-tumour immunity providing an immune shield to the tumour (7). PD-L1 over expression has been documented in various malignancies like melanomas, renal cell carcinomas, non-small cell carcinoma of the lung and squamous cell carcinoma of head and neck. In gastric cancer, it has been reported that PD-L1 over expression was found in 20 to 40% of gastric cancers, and these tumours are associated with advanced disease stage and/or worse clinical outcome (8). In the present study PD-L1 expression is studied on archival tissues from gastrectomy specimens and correlated with various clinicopathological features retrieved from the medical records. The effect of over expression of PD-L1 in tumour cells and tumour infiltrating immune cells on various

clinicopathological aspects of gastric carcinoma was studied.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the institutional ethics committee of Sri Ramachandra Institute of Higher Education and Research. This is a retrospective study done from 2014 to 2017 on paraffin blocks of 100 cases of primary gastric carcinoma who underwent curative resection without preoperative chemotherapy. Clearance from the institutional ethics committee was obtained prior to commencing the study. (REF: IEC NO: CSP-MED/16/OCT/31/145).

The clinical data of patients including age, gender and stage are obtained from the medical records section. The histopathological data is collected from the pathological case files. For each case, all available haematoxylin and eosin-stained sections are reviewed, and a representative block showing the tumour with margins is selected for immunohistochemical studies. Blocks with necrosis are excluded. Immunostaining is done using a qualitative immunohistochemical assay from Ventana Roche with rabbit monoclonal anti PD-L1 clone SP142 intended for use in the assessment of the PD-L1 protein in formalin -fixed, paraffin -embedded

(FFPE) tissue. The staining was performed on Automated IHC Stainer from Roche Ventana (Benchmark Ultra) as per the defined protocol.

Rabbit Monoclonal Negative Control immunoglobulin, a negative reagent control antibody specifically matched for this assay is run for every case and is used in place of the primary antibody to evaluate nonspecific staining. Normal human tonsil tissue is used as the control and is run on the same slide in every case. The presence of PD-L1 staining within the macrophages and lymphocytes in germinal centres and reticulated crypt epithelium of tonsil serve as positive tissue elements. Absence of staining in superficial squamous epithelium and negative immune cells in interfollicular regions of tonsil serve as negative tissue elements.

Assessment of PD-L1 expression in gastric carcinoma

Percentage of tumour cells with cytoplasmic or partial/complete membranous staining are enumerated. In areas with visible staining 200 cells are counted in 200x objective of the microscope and number of tumour cells positive is taken. Score more than 1% is considered as positive. Roche Ventana PD-L1 (SP142) assay scoring algorithm is used for the interpretation as described in table 1.

Table 1: Assessment criteria for tumour infiltrating lymphocytes

Immune cell (IC) stain assessment	PD-L1 Expression
Absence of any discernible PD-L1 staining (OR) Presence of discernible PD -L1 staining of any intensity in Tumour -infiltrating immune cells covering < 5% of tumour area occupied by tumour cells, associated intratumoral, and contiguous peritumoral stroma	< 5%
Presence of discernible PD -L1 staining of any intensity in tumour - infiltrating immune cells covering $\geq$ 5% of tumor area occupied by tumour cells, associated intratumoral, and contiguous peritumoral stroma	$\geq$ 5%

Statistical analysis

Statistical analysis of the data was performed by using the Chi Square and Fischer exact test. A predictive value of less than 0.05 is considered statistically significant.

RESULTS

Age of the study participants ranged from 25-85 years with a mean age of 57 years. Peak incidence is seen in the age group of 46-55 years. 65% are males and 35% are females with a male (65%) to female (35%) ratio of 1.8:1. Tumour site was divided as per the gross regions of stomach. Antrum is the most common site accounting for 70% of cases followed by body (20%), fundus (7%) and cardia (3%). Tumour size is found to be more than 4cm in 60 cases and 40 cases are < 4cm. With regard to the tumour configuration 78 cases showed ulcerated growth on gross examination and

the rest 22 cases showed exophytic growth (Figure 1a & 1b). On follow up 45 patients are alive, doing well of which 38 patients completed 6 cycles of chemotherapy, 39 patients succumbed to the disease. 16 patients are lost to follow up. Distant metastasis are identified in 4 patients and all 4 succumbed to the disease. Recurrence is noted in 2 cases. Table II shows comparison of 2 year survival with clinicopathological parameters. According to WHO histological grading system 4% cases are well differentiated, 53% moderately differentiated and 43% poorly differentiated. As per the Lauren's classification 57% cases are of intestinal type and 43% are diffuse type of adenocarcinoma (Figure 1c, 1d & 1e). All the cases are staged as per the TNM staging, 67 cases are stage III/IV and 33 cases are stage I/II. Lymphovascular invasion was identified in the H&E sections in 48 cases. Lymph node metastasis was observed in 63 cases.

Table 2: Correlation of tumour size, stage, lymphovascular invasion and lymph node involvement with survival Expression of PD-L1 in gastric carcinoma

	Clinical outcome			P value
	Living	Succumbed to disease	Lost to follow up	
Tumour size <4cm	19	12	9	
Tumour size > 4cm	26	27	7	< 0.001
Tumour stage I/II	17	10	6	
Tumour stage III/IV	28	29	10	<0.001
LVI present	18	24	6	
LVI absent	27	15	10	< 0.001
LN involved	27	27	9	
LN not involved	18	12	7	<0.001

Fig. 1: a) Gross Specimen of stomach showing ulceroproliferative growth. b) Gross Specimen of stomach with features of Linitis plastica. c) Microscopic image of Intestinal type of Adenocarcinoma Stomach (H&E;X40). d) Microscopic image of diffuse type of Adenocarcinoma Stomach (H&E;X100). e) Adenocarcinoma Stomach with intratumoral and peritumoral lymphocytes (H&E; X40)

PD-L1 expression is observed in tumour cells and immune cells but not in the adjacent non neoplastic gastric epithelium. In tumour cells positivity is considered as cytoplasmic or membranous staining of any intensity (Figure 2). As overall % of PD-L1 positive tumour cells is low, assessment of different

percentages of 3 different staining intensities is impractical and not done. Out of 100 cases 17% showed positivity for PD-L1 in tumour cells and 44% showed positivity for PD-L1 in immune cells (Figure 3).

Fig. 2: a) PD-L1 strong membranous and cytoplasmic staining in the tumor cells (IHC; X40). b) PD-L1 membranous staining of moderate intensity in the tumor cells (IHC;X200). c) PD-L1 membranous staining of mild intensity in the tumor cells (IHC;X200). d) PD-L1 staining in the intratumoural lymphocytes (IHC;X100).

Fig. 3: Percentage of PD-L1 positivity in tumour cells and tumour infiltrating immune cells

PD-L1 is positive in 9 cases in the age group of more than 60 years, while in patients less than 60 years 8 cases are found to be positive. Of the 17 positive cases 11 are males and 6 are females. As antrum is the most common site PD-L1 is predominantly positive in antral tumours. With regard to the tumour size 14 cases are found to be positive in patients with tumour size of more than 4cm while only 3 cases are positive in patients with tumour size of less than 4cm giving a significant correlation with a p value of 0.03. As per

the Lauren's histological classification 10 cases of intestinal type and 7 cases of diffuse type are positive for PD-L1. Among the 3 histological grades maximum positivity was noted in grade 2 cases (59%). With respect to the staging most positive cases fall in stage III/IV. 76% cases belong to stage III/IV whereas only 24% cases are stage I/II. PD-L1 is positive in 13 of 35 cases with lymphovascular invasion with a significant p value of 0.04. 13 of 50 lymph node positive cases are positive (Figure 4).

Fig. 4: Correlation of lymphovascular invasion with PD-L1 expression in tumour cells

Of the 17 PD-L1 positive cases on follow up 8 patients are alive and 9 succumbed to disease with a 2 year survival rate of 47%. In PDL1 negative cases 37 patients are alive, 30 succumbed to disease and 16 patients are lost to follow up. 2 year survival rate is 55% in PD-L1 negative cases in our study. PD-L1 expression in immune cells is considered positive with staining of any intensity seen in infiltrating immune cells covering >5% of tumour area. PD-L1 Expression in immune cells was positive in 44 cases, which showed a significant correlation with tumour location. Antral carcinomas showed more positivity with a p value of < 0.01. PD-L1 positivity in the immune cells is predominantly noted in cases with lymph node involvement compared to cases in which lymph nodes are negative with a significant p value of 0.012.

Tumour stage also shows a significant correlation with most positive cases falling under stage III/IV with a p value of 0.02. There was no statistical correlation between the expression of PD-L1 in immune cells and age, gender, tumour size, histological type, histological grade, lymphovascular invasion and survival. On follow up of the 44 cases that were positive for PD-L1 in immune cells, 23 patients were found to be doing well, 15 were dead and 6 patients were lost to follow up. 2 year survival rate in cases with PD-L1 positive immune cell was 60% whereas in PD-L1 negative cases 2 year survival rate was 47%. The correlation of PD-L1 expression in tumour cells and tumour infiltrating immune cells with clinicopathological parameters and survival is summarised in table III & IV.

Table 3: Correlation of PD-L1 expression in tumour cells and tumour infiltrating immune cells with various clinicopathological parameters

Subject variables	PD-L1 Expression in Tumour cells			PD-L1 Expression in Tumour Infiltrating immune cells		
	Positive (N=17)	Negative (N=83)	p Value	Positive (N=44)	Negative (N=56)	p Value
Age						
Less than 60 years	8	45	0.6	27	26	0.16
More than 60 years	9	38		17	30	
Gender						
Male	11	54	1	28	37	0.83
Female	6	29		16	19	
Tumour Location						
Antrum	13	57		26	44	0.00*
Body	3	17	0.53	13	7	
Fundus	0	7		3	4	
Cardia	1	2		2	1	
Tumour size						
Less than 4 cm	3	37	0.03*	13	27	0.06
More than 4 cm	14	46		31	29	
Histological type						
Diffuse	7	36	1	17	26	0.54
Intestinal	10	47		27	30	
Histological grade						
Grade I	2	2		27	30	0.54
Grade II	10	43	0.12	17	26	
Grade III	5	38		0	0	
Lymphovascular invasion						
Present	13	35	0.015*	25	23	0.16
Absent	4	48		19	33	
Lymph node involvement						
Present	13	50	0.275	34	29	0.012 *
Absent	4	33		10	27	
Tumour Stage						
Stage I/II	4	30	0.4	9	24	0.02 *
Stage III/IV	13	53		35	32	

Table 4: Correlation of PD-L1 Expression in tumour cells with survival

Survival	PD-L1 Expression in tumour cells			PD-L1 Expression in Tumour Infiltrating immune cells		
	Positive (N=17)	Negative (N=67)	p value (Fischer test)	Positive (N=38)	Negative (N=46)	p value (Fischer test)
Living	8	37	0.6	23	22	0.27
Succumbed to disease	9	30		15	24	

DISCUSSION

Gastric cancer is one of the most common cancers with majority presenting at an advanced stage. In India overall incidence of gastric carcinoma is low compared to other countries with highest incidence in Mizoram. In spite of the advancements in multimodality therapy, the prognosis and overall survival rate is low. The aggressiveness of the disease and need for improvement in therapeutic options is discerned by the fact that gastric cancer is the second most common cause of cancer death globally (9). This led to the development of novel strategies like immunotherapy. PD-L1 is one such immunomodulator whose expression has been observed in various solid tumours.

In the present retrospective study on 100 cases of gastric carcinoma we compared various clinicopathological parameters with the published literature and their association with expression of PD-L1. The mean age in our study is 57 years and prevalence is more common among males, in accordance with the study done by Sharma et al (10). In the study done by Yang et al, incidence of gastric carcinoma is high in > 75 years and low in < 44 years (11). However our study shows peak incidence in the age group of 46-55 years. Poorly differentiated tumours are more prevalent among < 60 years age group in contrast to well differentiated tumours and is in accordance with the study done by Bautista et al (12). This implies most of the poorly differentiated and aggressive tumours occur in younger age. Wang

HM et al has done a study on 430 patients of gastric carcinoma comparing the significance of tumour size with various clinicopathological parameters and overall survival. The mean tumour size was taken as 4.8cm and all the cases with tumour size of >4.8cm showed significant correlation with higher tumour stage, lymph node metastasis and poor 5 year survival rate (13). Our results are comparable to his study. Most of the tumours with serosal invasion and lymph node metastasis are more than 4cm compared to cases with no serosal invasion and lymph node negativity. Predominantly stage III/IV and poorly differentiated tumours are larger in size (>4cm). Tumour size also shows very significant correlation (p value- <0.001) with survival indicating it's an independent prognostic factor. Antrum is the most common site (70%) in our study and is in correspondence with the study done by Pinto-de-Sousa *et al.*, (14).

Based on Lauren's histological classification intestinal type (57%) is found to be predominant in our study compared to the diffuse type (43%). Most of the diffuse type show lymph node involvement and fell under TNM stage III/IV when compared to intestinal type. With concern to the histological grade, most of the tumours in our study are moderately differentiated (53%) and only 4% are well differentiated. In the study by Li *et al.*, 35.2% cases had lymphovascular invasion and showed significant correlation with the tumour size, type, differentiation, depth of invasion, lymph node metastasis, distant metastasis and survival (15). In our study lymphovascular invasion is noted in 48 cases and seen predominantly in poorly differentiated, lymph node positive and stage III/IV tumours. Regional lymph node metastasis is noted in 63 cases and correlated with the presence of lymphovascular invasion, higher tumour stage and survival. Most tumours fall in TNM stage III/IV category (67%) and almost all stage III/IV tumours are poorly differentiated. Lymphovascular invasion, lymph node metastasis, tumour stage and size show very significant correlation with survival with a p value of <0.001, each indicating that they are independent prognostic factors.

Expression of PD-L1 plays a key role in cancer immune escape and associated tumour progression and prognosis. PD-L1 overexpression has been observed in various solid cancers. It has already been proven in melanoma, non-small cell lung carcinoma and urothelial carcinoma. Patients with positive staining can be benefitted by targeted therapy against PD-L1 with highly promising results (16). The present study on PD-L1 expression in gastric carcinoma with clone SP 142 is the first evaluation in the Indian population. 100 cases of gastric carcinoma who underwent gastrectomy are studied for PD-L1 expression on the tumour cells, adjacent normal mucosa and tumour infiltrating immune cells. The cases showing positivity are evaluated for any

association with various clinico-pathological features. The expression of PD-L1 is found in cytoplasm and membrane, this pattern is consistent with previous reports. In our study PD-L1 expression is slightly higher in age group of more than 60 years and increased expression is noted among males compared to females. Kawazoe *et al* demonstrated statistically significant PD-L1 expression with respect to age and gender. However in our study we did not get any significant correlation with age and gender (17). This might be due to limited study candidates and ethnical factors. Tumours more than 4cm in size show increased PD-L1 expression compared to the tumours less than 4cm in size with a significant p value of 0.03 and is correlating with the studies done by Wu *et al.*, and Zhang *et al.*, (18, 19). In our study antrum is found to be the most common site of gastric carcinoma and shows statistically significant correlation with PD-L1 expression in immune cells but not with tumour cells. In studies done by Zhang *et al.*, there is no significant correlation with the location of the tumour (19). Increased expression of PD-L1 is noted in intestinal type similar to the studies done by Kawazoe *et al* and Satio *et al.*, (17, 20). However there is no statistically significant correlation. Most of the PD-L1 positive tumours show moderately differentiated adenocarcinoma but there is no statistical correlation between histological grade and PD-L1 expression. Tamura *et al.*, did a study on 431 patients of which 128 showed PD-L1 positivity and showed significant correlation with lymphovascular invasion, lymph node metastasis and tumour stage. In the current study also there is a significant correlation with lymphovascular invasion with a p value of 0.015 (20).

In the current study PD-L1 positivity in immune cells correlated with tumour stage with a p value of 0.02. This is in accordance with studies done by Satio *et al.*, & Boger *et al.*, whereas Kawazoe *et al.*, did not find significant correlation. However positivity in tumour cells did not show significant correlation (17, 20, 21). Boger *et al* also found that PD-L1 positivity in tumour infiltrating immune cells significantly correlated with lymph node involvement (21). Our study also shows similar results with a significant p value of 0.012. On the contrary Eto *et al.*, did not find any significant correlation with lymph node involvement (22). Several studies have demonstrated that PD-L1 expression plays a key role in cancer immune escape, tumour progression and indicates a poor prognosis. These highlighted studies demonstrated that PD-L1 may serve as a potential prognostic and predictive biomarker. However for patients with gastric carcinoma, the association between expression of PD-L1 and their prognosis remains controversial. Few studies have demonstrated a significantly poor prognosis with positive PD-L1 expression (21-23), but other studies showed favourable survival outcomes (24). In our study 2 year survival rate in PD-L1 positive cases is slightly lower when compared to the

negative cases. In cases with immune cell positivity survival is better in PD-L1 positive cases. However both did not show significant correlation. Also taking into account the significant correlation of PD-L1 positivity with increased tumour size, presence of lymphovascular invasion, high tumour stage and lymph node involvement, PD-L1 positivity may be associated with poor prognosis. The correlation between PD-L1 expression and poor prognosis can be explained by the weakening of immunogenicity and escape from the anti-tumour immune response upon PD-L1 expression by tumour cells. PD-L1 expression is an independent prognostic predictor in gastric carcinoma as per the studies done by Boger C et al (21). In a meta-analysis by Zhang *et al.*, PD-L1 expression in gastric cancer is associated with a shorter overall survival indicating that it is a valuable prognostic predictor (23). Limitations in our study are definition of PD-L1 positivity, i.e. determination of cut off values for percentage of stained cells were difficult and bias in the selection of patients as this is a retrospective study. Follow up could not be collected for all the cases. The positive rate of PD-L1 in gastric cancer patients varied in different studies and also depends on the clone used. The clone used in the present study is Ventana SP142 assay which showed lesser positivity when compared to other studies with different clones. In 2015 a workshop was conducted by FDA on 4 PD-L1 assays (Dako 22C3, 28-8, Ventana SP263 and SP142) in non-small cell lung carcinoma which showed fewer positive tumour cells with clone SP 142 compared to the others. This might be the reason for low positivity of PD-L1 in the present study. Studies done by Zheng et al and Kawazoe et al using SP142 assay also showed lesser positivity similar to our study (4, 17). PD-L1 expression is considered as a predictive biomarker for tumour response to PD-L1 antibody treatment. Pembrolizumab phase 1 trials have been successful in treating lung carcinoma and melanoma. PD-L1 monoclonal antibody is currently undergoing clinical trials for advanced gastric carcinoma (25).

CONCLUSION

Present study shows substantial expression of PD-L1 in patients with gastric carcinoma. Increased expression is noted in tumour infiltrating immune cells than the tumour cells. Our results indicate that positivity of PD-L1 is related to larger tumour size, presence of lymphovascular invasion, lymph node metastasis and higher tumour stage with statistical significance. However PD-L1 expression cannot be demonstrated as an independent prognostic factor. PD-L1 immunohistochemistry can be potentially helpful in screening candidates for anti PDL1 therapy. Well-designed multicentre cohort studies in the Indian population are needed to confirm these findings.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest among authors for this study.

REFERENCES

- Shrikhande, S. V., Sirohi, B., Barreto, S.G., Chacko, R.T., Parikh, P. M., Pautu, J., *et al.*, Indian Council of Medical Research consensus document for the management of gastric cancer. *Indian J Med Paediatr Oncol.* 2014 Oct; 35(4): 239-243.
- Nagini, S. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. *World J Gastrointest Oncol.* 2012; 4(7): 156.
- Sitarz, R., Skierucha, M., Mielko, J., Offerhaus, G. J. A., Maciejewski, R., Polkowski, W. P. Gastric cancer: epidemiology, prevention, classification, and treatment. *Cancer Manag Res.* 2018; 10: 239-248.
- Zheng, P., Zhou Z. Human Cancer Immunotherapy with PD-1/PDL1 Blockade. *Biomark Cancer.* 2015; 7(Suppl 2): 15-18.
- Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., *et al.*, PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. *Front Pharmacol.* 2017; 8: 561.
- Tamura, T., Ohira, M., Tanaka, H., Muguruma, K., Toyokawa, T., Kubo, N., *et al.*, Programmed death-1 ligand-1 (PDL1) expression is associated with the prognosis of patients with stage II/III gastric cancer. *Anticancer Res.* 2015 Oct 1; 35(10): 5369-5376.
- Jiang, Y., Li, Y., Zhu, B. T-cell exhaustion in the tumour microenvironment. *Cell Death Dis.* 2015 Jun 18; 6(6): e1792-e1792.
- Gu, L., Chen, M., Guo, D., Zhu, H., Zhang, W., Pan, J., *et al.*, PD-L1 and gastric cancer prognosis: A systematic review and meta-analysis. *Kato M, editor. PLoS One.* 2017 Aug 10; 12(8): e0182692.
- Alberts, S. R., Cervantes, A., van de Velde, C. J. H. Gastric cancer: epidemiology, pathology and treatment. *Ann Oncol Off J Eur Soc Med Oncol.* 2003; 14 Suppl 2: ii 31-36.
- Sharma, A., Radhakrishnan, V. Gastric cancer in India. *Indian J Med Paediatr Oncol.* 2011 Jan; 32(1): 12-16.
- Yang, D., Hendifar, A., Lenz, C., Togawa, K., Lenz, F., Lurje, G., *et al.*, Survival of metastatic gastric cancer: Significance of age, sex and race/ethnicity. *J Gastrointest Oncol.* 2011 Jun; 2(2): 77-84.
- Bautista, M. C., Jiang, S. F., Armstrong, M. A., Postlethwaite, D., Li, D. Impact of age on clinicopathological features and survival of patients with noncardia gastric adenocarcinoma. *J Gastric Cancer.* 2014 Dec; 14(4): 238-245.
- Wang, H. M., Huang, C. M., Zheng, C. H., Li, P., Xie, J. W., Wang, J. B., *et al.*, Tumour size as a prognostic factor in patients with advanced gastric cancer in the lower third of the stomach. *World J Gastroenterol.* 2012 Oct 14; 18(38): 5470.
- Pinto-de-Sousa, J., David, L., Seixas, M., Pimenta, A. Clinicopathologic profiles and prognosis of gastric carcinomas from the cardia, Fundus/Body and Antrum. *Dig Surg.* 2001; 18(2): 102-110.
- Li, P., He, H. Q., Zhu, C. M., Ling, Y. H., Hu, W. M., Zhang, X. K., *et al.*, The prognostic significance of lymphovascular invasion in patients with resectable gastric cancer: a large retrospective study from Southern China. *BMC Cancer.* 2015 Dec 7; 15(1): 370.
- Wu, P., Wu, D., Li, L., Chai, Y., Huang, J. PD-L1 and survival in solid tumours: a meta-analysis. *Soutto M, editor. PLoS One.* 2015 Jun 26; 10(6): e0131403.
- Kawazoe, A., Kuwata, T., Kuboki, Y., Shitara, K., Nagatsuma, A. K., Aizawa, M., *et al.*, Clinicopathological features of programmed death ligand 1 expression with tumour-infiltrating lymphocyte, mismatch repair, and Epstein-Barr virus status in a large cohort of gastric cancer patients. *Gastric Cancer.* 2017 May 14; 20(3): 407-415.

18. Wu, C., Zhu, Y., Jiang, J., Zhao, J., Zhang, X.G., Xu, N. Immunohistochemical localization of programmed death-1 ligand1 (PD-L1) in gastric carcinoma and its clinical significance. *Acta Histochem* [Internet]. 2006 May [cited 2018; 108(1): 19-24.
19. Zhang, L., Qiu, M., Jin, Y., Ji, J., Li, B., Wang, X., *et al.*, Programmed cell death ligand 1 (PD-L1) expression on gastric cancer and its relationship with clinicopathologic factors. *Int J Clin Exp Pathol*. 2015; 8(9): 11084-11091.
20. Saito, R., Abe, H., Kunita, A., Yamashita, H., Seto, Y., Fukayama, M. Overexpression and gene amplification of PD-L1 in cancer cells and PD-L1+ immune cells in Epstein-Barr virus-associated gastric cancer: the prognostic implications. *Mod Pathol*. 2017; 30(3): 427-439.
21. Böger, C., Behrens, H. M., Mathiak, M., Krüger, S., Kalthoff, H., Röcken, C., *et al.*, PD-L1 is an independent prognostic predictor in gastric cancer of Western patients. *Oncotarget*. 2016 Apr 26; 7(17): 24269-242683.
22. Eto, S., Yoshikawa, K., Nishi, M., Higashijima, J., Tokunaga, T., Nakao, T., *et al.*, Programmed cell death protein 1 expression is an independent prognostic factor in gastric cancer after curative resection. *Gastric Cancer* [Internet]. 2016; 19(2):466-471.
23. Zhang, M., Dong, Y., Liu, H., Wang, Y., Zhao, S., Xuan, Q., *et al.*, The clinicopathological and prognostic significance of PD-L1 expression in gastric cancer: a meta-analysis of 10 studies with 1,901 patients. *Sci Rep*. 2016; 6:37933.
24. Kim, J. W., Nam, K. H., Ahn, S. H., Park, D. J., Kim, H. H., Kim, S. H., *et al.*, Prognostic implications of immunosuppressive protein expression in tumours as well as immune cell infiltration within the tumor microenvironment in gastric cancer. *Gastric Cancer*. 2016 Jan 26; 19(1): 42-52.
25. Zou, W., Wolchok, J. D., Chen, L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. *Sci Transl Med*. 2016 Mar 2; 8(328): 328rv4.