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Expression of Programmed Death Ligand (PD-L1) in Ovarian Surface Epithelial Tumours

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ABSTRACT

Introduction: Malignant surface epithelial ovarian carcinomas usually present at an advanced stage of the disease and also one of the leading causes of cancer-related deaths in women despite multimodality treatment. Programmed death-ligand -1 (PDL-1) is a co-regulatory molecule expressed on the surface of ovarian tumour cells as well as immune cells, binds to its receptor programmed death receptor (PD-1) and suppresses the local tumour immunity thereby helping the tumour cells to spread and metastasize.

Objective: To determine the immunohistochemical expression of PD L-1 in tumour cells and immune cells in malignant surface epithelial tumours of the ovary. To correlate the level of PDL-1 expression with histopathological parameters of surface epithelial tumours of the ovary.

Methods: Immunohistochemical expression of PDL-1 in 100 cases of surface epithelial tumours of ovary were studied. Clinicopathological parameters, history of presurgical treatment, response to treatment, presence of tumour implants and presence of metastasis etc were obtained from the hospital medical records. The intensity and extent of membranous staining by anti-PD L-1 were scored for the tumoural cells and the tumour infiltrating immune cells separately. Statistical analysis was done on the data collected using the "SPSS Version 11" statistical program. Pearson Chi-square test was used to determine significant clinicopathological differences between PD-L1 expression in positive and negative tumours.

Results: PDL-1 expression in tumour cells was high in 6(6%) cases and 22(22%) cases showed expression in immune cells in tumour micro-environment. A significant statistical correlation between PDL-1 expression and grade and histological type of tumour was noted.

Conclusion: High expression of PD-LI was noted in tumour infiltrating immune cells in more number of cases than that of the expression in tumour cells. The present study indicates that expression of PD-L1 is related to histologic type, the grade of the tumour and ovarian surface involvement by tumour cells with statistical significance.

Key Words: PDL-1, Immunohistochemistry, Ovarian surface epithelial tumour, Immune cells, Immunotherapy, Ovarian cancer

INTRODUCTION

Malignant surface epithelial ovarian carcinomas are one of the leading causes of cancer-related deaths in women among the gynaecological malignancies. Many of these patients present at an advanced stage of the disease and therefore succumb to it despite multimodality treatment.[1] Programmed death ligand 1 (PD-L1) is a co-regulatory molecule that is expressed on the surface of various type of cells including immune cells and epithelial cells. By binding to its receptor, programmed death receptor 1 (PD-1) on lymphocytes it generates an inhibitory signal toward T-cell receptor-mediated activation of lymphocytes. Ovarian cancer cells express PD-L1 upon encountering immune cells and as a consequence inhibit cytotoxic T-lymphocyte function, escape from them and disseminate[1]. PD-L1 expression by ovarian tumour cells is seen to be associated with malignant morphology as compared with borderline tumours.[2,3]

Targeted therapies are now available to block the interaction between PD-L1 and PD-1 to prevent the spread of cancer cells. Therefore, it is important to evaluate the expression

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of PD-L1/PD-L1 in tumour cells as well as immune cells to get benefited from these targeted therapies. In our study, we have evaluated the expression of PD-L1 and PD-1 in tumour cells and immune cells in malignant surface epithelial tumours of the ovary.[4]

MATERIALS AND METHODS

This is a retrospective study done in the Department of Pathology at Sri Ramachandra Institute of Higher Education and Research over 4 years from January 2015 to December 2018. A total of 116 surgical resection specimens of borderline and malignant surface epithelial tumours of ovary were received during the period of which blocks were not available or with insufficient tissues in 16 cases. So a final number of 100 cases were retrieved and reviewed. Permission from the Institutional Ethics committee was obtained before commencing the study (IEC No CSP-MED/18/JAN/41/04). Patient details including age, tumour location, tumour laterality, history of presurgical treatment, response to treatment, presence of tumour implants and presence of metastasis etc were obtained from the medical records section.

Representative sections from formalin-fixed paraffin-embedded blocks were used for this study. Hematoxylin & eosin stained 3-5 microns thin sections were retrieved and reviewed. The slides were then analysed for the type of tumour tissue and the presence of tumour infiltrating immune cells. One representative section containing the tumour tissue and tumour infiltrating immune cells was chosen for each case for the immunohistochemical study.

The immunohistochemical study of Programmed death ligand-1 (Antibody Type: Rabbit monoclonal Anti-CD 274 antibody, Clone: RBT-PDL1, Isotype: IgG, Source: Rabbit, Immunogen: Synthetic peptide corresponding to N-terminus residues of human PD-L1 protein, Localisation: Membranous, Reactivity: Paraffin, frozen tissues) from BIO SB. Inc, Santa Barbara, USA) was carried out manually. Tissue from tonsil and placenta was used as positive controls. A non-immunological serum replacing the primary antibody was used as a negative control.

All slides were then examined and scored with a template. Only membranous staining by anti-PD L-1 was considered as positive and cytoplasmic staining was considered as negative in this study. The intensity and extent of membranous staining by anti-PD L-1 were scored for the tumoural cells and the tumour infiltrating immune cells separately. The staining intensity was scored as 0 (negative) to 3 (strong). The extent of staining was scored as 0 (<5%), 1 (5-25%), 2 (26-50%), 3 (51-75%), 4 (>75%), according to the positive staining areas concerning the whole tumoural area. Scores for staining intensity and percentage positivity of cells were then multiplied to get the immunoreactivity score (IRS) for each case. Samples having a final staining score of ≤ 4 were considered to have low expression and those with a score of >4 were considered to have high expression.

Statistical analysis

This was done on the data collected using the "SPSS Version 11" statistical program. Pearson Chi-square test was used to determine significant clinicopathological differences between PD-L1 expression in positive and negative tumours. Differences were considered statistically significant when the P-value was < 0.05

RESULTS

A total number of 100 cases were studied, out of which 23 were borderline tumours and 77 were malignant surface epithelial tumours. Among the malignant tumours, serous carcinoma was the most common one comprising of 71% (55 out of 77 cases). The age distribution of the patients was from 17 to 85 years with the median age at 50 and the maximum number of cases from 45 to 60 years of age (41%). 59% of cases were postmenopausal. Unilateral tumours (72%) were common than bilateral tumours (28%). Ovarian surface involvement and presence of peritoneal implants were seen in 18% and 21% of cases respectively. Ascitic fluid was positive in 11 cases and lymph node metastasis at the time of presentation was seen in 16 cases. It was noted that 42% of cases were at stage I at the time of presentation given the fact that borderline cases (23 in number) were also included in our study. Among the malignant tumours majority patients presented at stage III. Pre-operative adjuvant therapy was given in 35 cases and the surgical specimens were assessed for treatment response. The marked response was seen in 8 cases, the moderate response in 14 cases, minimal response in 11 cases and no response in 2 cases.

Membranous expression of PD-L1 has assessed in the tumour as well as the immune cells surrounding or infiltrating the tumour in all the 100 cases (Fig.1,3). High expression of PD-L1 in tumour cells (Fig.1) was seen only in 6 out of the total number of cases, 13 cases showed low expression and the remaining 81 cases were negative for PD-L1. High expression of PD-L1 in the immune cells are seen in 22 cases (Fig.2,3), 8 cases showed low expression and the remaining 70 cases showed no expression of PD-L1. A statistically significant correlation of PD-L1 expression in tumour cells and immune cells was seen in association with the histological type of tumour (serous carcinomas showed higher expression than other tumours) with a p-value of 0.009 (for expression in tumour cells) and 0.004 (for expression in immune cells) respectively, with histological grade of the tumour (p-value 0.034 and 0.008 respectively).

High expression of PD-L1 in tumour cells alone was noted

insignificant statistical correlation with the presence of ovarian surface involvement with a p-value of 0.018. This was not seen with expression in immune cells. However high expression of PD-L1 in immune cells were noted more in malignant cases (fig. 1) than borderline tumours with a statistically significant correlation and a p-value of 0.026. The correlation between PD-L1 expression in tumour cells and immune was also found to be significant in our study with p-value 0.001.

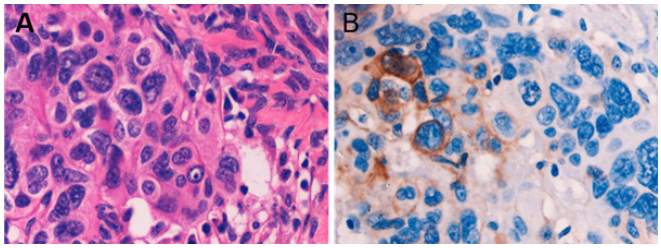

Figure 1: A. Tumour cells with anisonucleosis and irregular nuclear contours, coarse nuclear chromatin, occasional prominent nucleoli and scant to moderate eosinophilic cytoplasm. (haematoxylin and eosin stain; magnification x 400). B. Immunohistochemistry: 3+ Membranous positivity of PD-L1 in tumour cells (magnification x 400)

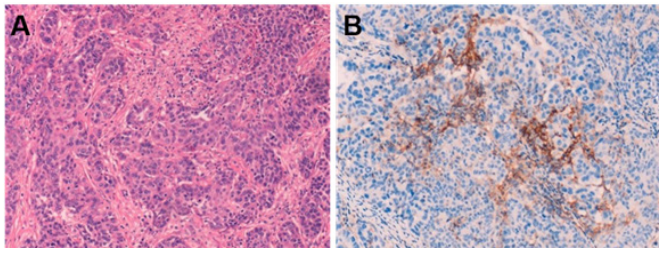

Figure 2: A. Tumour nest and focal syncytium pattern. (haematoxylin and eosin stain; magnification x 100). B. Immunohistochemistry: PD-L1 positivity in the immune cells in tumour micro-environment. The tumour cells are negative for PD-L1 (Immunohistochemistry; magnification x 100)

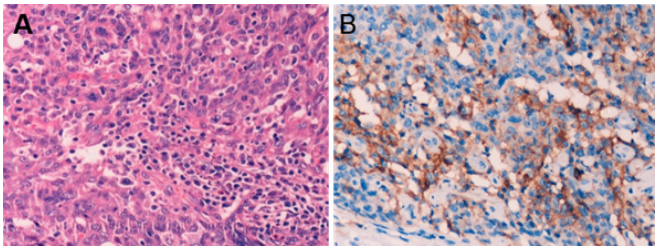

Figure 3: A. Tumour arranged in syncytium pattern. (haematoxylin and eosin stain; magnification x 100). B. Immunohistochemistry: Positive PD-L1 expression in both the tumour cells as well as the immune cells. (Immunohistochemistry; magnification x 200).

DISCUSSION

Ovarian tumours are one of the most lethal gynaecological malignancies with a significant number of patients presenting at an advanced stage of the disease. Despite the advancements in multimodality therapy, the prognosis and overall survival rate of high-grade ovarian carcinomas are considerably low.[3] The aggressiveness of the disease and the need for therapeutic improvement in these malignancies is recognized by the fact that ovarian tumours are the principal cause of cancer-related deaths among the gynecologic malignancies. This had led to the development of novel therapeutic strategies such as immunotherapy. PD L-1 is one among the immunomodulators whose expression has been observed in a variety of solid tumours.[4] PD-L1 expression in tumour cells and in the immune cells surrounding and infiltrating the tumour plays an important role in the immune evasion of tumours and is associated with tumour progression in a variety of solid tumours like melanoma, small cell carcinoma of the lung and urothelial carcinoma, where the patients with positive staining can be benefited by targeted treatment against PD-L1.[5]

A wide age range is noted in the present study with the youngest patient at 17 years of age and the eldest at 85 years. The mean age is 50 years and the incidence is more common in postmenopausal women with a peak incidence in the age group of 45 to 60 years of age. Out of the 100 patients, 13 were nulliparous, one is a primigravida and the remaining 86 cases are multiparous. Majority of the tumours are unilateral (72%) which is slightly higher than was observed by Shirish et al[6], with a little predilection towards the right side tumour (40%) than the left side (32%). The incidence of bilateral tumours is 28% similar to a earlier study.[7] Majority of our patients presented at stage I disease (57%), which is in contrast with the study done by Tingulstad et al. However, we attribute this disparity to the inclusion of borderline tumours in our study population. The size of the tumours was compared with other clinicopathological parameters. The tumours were grouped according to their size, those measuring less than 5 cm in size, between 5 to 10 cm in size and tumours more than 10 cm in size. There is a significant statistical correlation between the size of the tumours with the presence of metastatic lymph node and the stage of the disease with a p-value of 0.015 and 0.000 respectively.

In the present study, the most common histological type of ovarian surface epithelial tumours is serous carcinoma with 54% of the cases followed by mucinous borderline tumours and is in concordance with a previous study.[8] Undifferentiated carcinoma has the least incidence seen in only one patient out of 100 and the similar result has been observed in a study done by Nageswara Rao et al. Regarding the grades of the tumours, high-grade tumours are more common (52 out of 54 cases of serous carcinomas and 6 out of 9 cases

of endometrioid carcinomas were of high grade) than low-grade tumours among the malignant tumours. Ascitic fluid/peritoneal wash cytology was studied in all the 100 cases out of which 11 cases showed the presence of malignant cells. Serous carcinomas revealed a higher frequency of positive peritoneal cytology with 10 cases out of 11. A significant statistical correlation of positive cytology with omental metastasis and capsular invasion is also reported. However, these correlations with omental metastasis capsular invasion were not seen in our study.

The presence of lymphovascular invasion showed a significant correlation with the stage of the disease and nodal involvement with a p-value of 0.018 and 0.015 respectively. In our study, expression of PD- L1 has been evaluated in tumour cells as well as in immune cells in surface epithelial tumours of the ovary. Strong membranous staining of cells is taken as positive and the positive cases were evaluated for any association with various clinicopathological features.

In this study, the expression of PD-L1 in the tumour, as well as immune cells, is slightly higher in patients with age more than 45 years of age, however, this was not statistically significant. This may be because of the limited study population and selection bias. Overall, high expression of PD-L1 in tumour cells is seen only in 6 cases out of 100. Similar incidence has been seen in a study done by Dennis et al with only 3.5% cases showing high expression of PD-L1 in tumour cells. However, the percentage of cases showing low expression of PD-L1 is only 13% in our study, which is significantly lower than theirs with 33.2%.[9]

High expression of PD-L1 in tumour cells is noted more in malignant tumours (83.3%)rather than borderline tumours (5 out of 6 cases with high expression of PD-L1 in tumour cells are malignant tumours). This is in correlation with a previous study.[10] However, we did not find this to be statistically significant (p-value = 0.284). High PD-L1 expression in tumour cells is noted with increased frequency in serous carcinomas than the other histologic tumour types of ovarian surface epithelial neoplasms with a significant statistical correlation (p= 0.005). There is a higher predilection towards the high-grade tumours with a p-value of 0.034 similar to earlier study.[11]

Ovarian surface involvement by the tumour cells is found to have a significant correlation with expression of PD-L1 in tumour cells having a p-value of 0.018. However PD-L1 expression in tumour cells was not correlated with other clinicopathological parameters like age of the patient, stage of the disease, nodal involvement, tumour implants, chemotherapy and residual tumour burden as seen in the study done by Hamanishi et al.[12] Regarding the correlation of PD-L1 expression in tumour cells with expression in immune cells, all the cases which showed high expression of PD-L1 in tumour cells were also showing high expression in the immune cells with a significant statistical correlation (p-value = 0.002).

High expression of PD-L1 in immune cells surrounding or infiltrating the tumour was noted in 22% of the cases which is significantly higher than the number of cases with high PD-L1 expression in tumour cells (only 6%) similar to a study done by Webb et al. Low expression of PD-L1 in immune cells was seen in 8% of the cases and the remaining 80% cases were negative for PD-L1 expression.

High expression of PD-L1 in immune cells is seen in more number of malignant cases than borderline cases (20 out of 22 cases with high expression in immune cells are malignant, 90.9%) and was found to be statistically significant with a p-value of 0.026. This is in correlation with the study done by Kahraman et al.[10]

High expression of PD-L1 in immune cells was seen in increased frequency with serous carcinomas than other histological subtypes (p=0.004) and also seen an insignificant correlation with high-grade tumours (p=0.008) similar to the study by Webb et al.[11] High expression of PD-L1 in immune cells was seen in more numbers in stage I tumours than higher staged tumours, however, this was not statistically significant. There was also no significant statistical correlation of PD-L1 expression in immune cells with other clinicoapthological parameters like age of the patient, menopausal status, size of the tumour, presence of lymphovascular invasion, ovarian surface involvement, lymph node involvement and presence of malignant cells in ascitic fluid/peritoneal wash cytology.

CONCLUSION

This study shows the substantial expression of PD-L1 in patients with surface epithelial tumours of the ovary. High expression of PD-LI was noted in tumour infiltrating immune cells in more number of cases than that of the expression in tumour cells. Present study indicates that expression of PD-L1 is related to histologic type, grade of the tumour and ovarian surface involvement by tumour cells with statistical significance. However, the value of PD-L1 expression as an independent prognostic factor could not be demonstrated in our study. Nevertheless PD-L1 immunostaining can be used as a potential tool in screening candidates for anti-PD-L1 immunotherapy.

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