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PD-L1 Expression in the Epithelial Ovarian Carcinoma

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ABSTRACT

Introduction :
Ovarian cancer is one of the most lethal cancers amongst the females, and ranks as fifth most common cancer causing mortality worldwide.Around 80% of ovarian cancers epithelial in origin and originate from the surface epithelium .Various Studies have shown that the expressions of programmed death ligand-1 (PD-L1) and PD-L2 can be detected in a variety of tumor tissues, such as lung carcinoma, squamous cell carcinoma, breast cancer and liver cancer, providing an important foundation for studying mechanisms underlying the Ovarian carcinoma.In present study author studies PD-L1 Expression in the Epithelial Ovarian carcinoma.

Materials and methods : PD-L1 IHC was applied to all cases of ovarian tumors diagnosed as epithelial ovarian carcinoma on histopathological examination from year 2015 to 2022 in Department of pathology. Only membranous staining by anti-PD L-1 was considered as positive and cytoplasmic staining was considered as negative in this study.

Results : In present study it was seen that Malignant Serous Epithelial carcinoma were the most common lesion found. The expression of PD-L1 was examined in the immune stained slides of epithelial ovarian neoplasm, and the intensity and extent of membranous staining by anti – PD L-1 were examined. Out of the 84 cases none of thecases showed intense positivity, fair positivity and weak or focal positivity. Overall, 100 % cases showed PD-L1 negative expression.

Conclusion :
PD-L1 IHC is studied in many tumors but current study stated negative PD-L1expression in cases of ovarian epithelial neoplasm.

Keywords : ovarian cancer, epithelial ovarian tumor, IHC , PD-L1, membrane positivity, transmembrane glycoprotein.

I. INTRODUCTION

Ovarian cancer is one of the most lethal cancers amongst the females, and ranks as fifth most common cancer causing mortality worldwide. The incidence rate is higher in white women followed by Hispanic, Asians, Black and American Indian women [(1).] In India, the Age Standardized incidence Rate (ASR) for ovarian Carcinoma varied from 0.9 to 8.4 per 1,00,000 person/year[2]. Studies revealed that the peak incidence is in between the age of 55-64 years. The mean annual percentage increase in ASR ranges from 0.7 to 2.4%. [(2)]Around 80% of ovarian cancers epithelial in origin and originate from the surface epithelium .

Various Studies have shown the expressions of programmed death ligand-1 (PD-L1) and PD-L2 can be detected in a variety of tumor tissues, such as lung carcinoma, squamous cell carcinoma, breast cancer and liver cancer, providing an important foundation for studying mechanisms underlying the Ovarian carcinoma [(3).]

PD-L1 is a type I transmembrane glycoprotein expressed on activated T and B cells, DCs, macrophages, mesenchymal stem cells, and bone marrow derived mast cells[(4)(5).] It is also expressed on a wide variety of non-hematopoietic cells including the vascular endothelium, fibroblastic reticular cells, keratinocytes, lung, nonparenchymal cells of the liver, mesenchymal stem cells, pancreatic islet cells, astrocytes, and neurons.[(5,6)]

PD-L1 expression on human T cells, is induced by common γ chain cytokines (IL-2, IL-7, and IL-15), whereas PD-L1 expression on B cells is stimulated by IL-21 [(4).] In cancer cells, PD-L1 expression is regulated by the MAPK and PI3K/AKT pathways, as well as by HIF-1α, STAT-3, NF-κB and epigenetic mechanisms via micro RNAs.[(6)]

In present study author studies PD-L1 Expression in the Epithelial Ovarian carcinoma.

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Aim and Objective

Aim : To evaluate expression of PD- L1 In tumour tissue of epithelial ovarian carcinoma.

Objective :

To correlate relationship between their expression, clinicopathological findings and discuss prognostic value of PD- L1 in ovarian carcinoma.

Material and Methods

Type of Study: Observational, Cross Sectional and Descriptive study

Duration of Study

- From January 2015 to December 2019 retrospectively.
- From November 2020 to September 2022 prospectively.

Place of Study: Department of Pathology, Institute of Medical Science (IMS), BHU

Sample Size: 84 cases

Sampling Method : Convenient Sampling

Selection of cases

Submitted to the Department of Pathology from the patients attending operation theatre in Department of Obstetrics and Gynaecology of IMS, BHU during the study duration.

Inclusion Criteria: All cases of ovarian tumors diagnosed as epithelial ovarian carcinoma on histopathological examination from year 2015 to 2022 in Department of pathology.

Exclusion Criteria: Ovarian tumors other than epithelial ovarian carcinoma.

Histopathological examination :

The H&E sections of all the cases were examined by pathologist, detailed and systematic morphological evaluation was done and an appropriate section was selected for IHC examination. Immunohistochemical study was carried out using anti-PD-L1 specific antibody with following specifications :

Antibody	Clone	Dilution	Company	Positive control	Positivity
Rabbit monoclonal antibody in PBS	IHC 411	Ready to use	Biogenex	SCLC or tonsil	Membranous

Secondary antibody included : Polymer – HRP Anti -mouse and rabbit enhancer Reagent Company- Medaysis(Ready to use).

STEPS OF IHC

Thin sections of 2 to 4 micron were cut from formalin fixed paraffin embedded biopsy samples and mounted on Poly- L lysine coatedglass slides, followed by incubation for 1 hour at 60 -62 degree Celsius. Slides were then dewaxed in xylene for 10-15 minutes and blotted to remove excess xylene. Further rehydration was done at 100 %, 90%, 70% alcohol for 1-2 minute each, followed by antigen retrieval done in 2 cycles in Tris EDTA, first at 95 and second at 97 degree Celsius in buffer with PH 9.0.Slides were then left to cool at room temperature and were washed with Tris buffer { ph= 7.6} three times, 5 minutes each. After which slides were incubated in 0.5 % H_20_2 for 10 to 15 minutes (to block endogenous peroxidase activity). Then the slides were washed thoroughly with phoshphate buffer saline and incubated in primary antibody {PD-L1}, for 1 hr at room temperature followed by washing and dipping in super-enhancer for 10 minutes and distilled water for 2-3 dips.

Slides were kept in Horseradish peroxidase { HRP} for 10 minutes and washed in phosphate buffer saline. The section was then developed in 0.01% 3,3 diamino benzamidine tetrahydrochlroide {DAB} containing 0.003 % H_2O_2 in 10 mmol imidazole / PBS for 20 minutes, washed in PBS and deionized water and counter-staining was done with hematoxyline and then mounting.

Interpretation OF PD- L1

Only membranous staining by anti-PD L-1 was considered as positive and cytoplasmic staining was considered as negative in this study.

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 tumoral 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.**

Observations

The present study was conducted in the Department of Pathology and Obstetrics and Gynecology, IMS,BHU Varanasi. Total number of cases

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recorded during the study period were 84, out of which , 16 were borderline and 68 were malignant.

Table 1 : Histological Distribution of Ovarian Epithelial Carcinomas

Types/Group of cases	Total No. of cases	Percentage (%)
Borderline mucinous	12	14.3 %
Borderline serous	5	5.6 %
Malignant serous	34	40 %
Malignant mucinous	13	15.5 %
Malignant brenner's	4	4.8 %
Clear cell carcinoma	4	4.8 %
Endometroid	8	9.6 %
Transistional	1	1.2 %
Undifferentiated	1	1.2 %
Seromucinous	2	2.4 %
Total	84	100

As stated in Table 1 , malignant Serous Epithelial carcinoma was the most common lesion found followed by malignant mucinous carcinoma.

2: Age wise distribution of cases

In this study, the age distribution of ovarian carcinomas were ranging from 15 to 85 years and it was seen that the highest incidence of borderline tumors were among in the age group 56-65 years (3/10 cases, 30 %). The malignant tumors were common in the age groups 36-45 years (19/74 cases, 25 %).

Table No. 3 : Clinical Correlation

S. No.	Clinical Features	NO OF CASES	PERCENTAGE
1.	Abdominal mass	78	92 %
2.	Pain abdomen	4	4.8 %
3.	Ascites, peritoneum involvement	2	2.38 %
4	Asymtomatic	1	1.2 %

As seen from above table non. 3 Abdominal mass was the most common clinical presentation (78/84,92 %) followed by pain abdomen (4/84, 4.8%).

Table 4: Gross Examination findings

S.No.	Gross Morphology	No. of cases	Percentage
1.	Purely cystic	52	61 %
2.	Mixed solid and cystic	19	22.7 %
3.	Purely solid	7	8 %
4.	Cystic with papilary excrescences	5	5.95 %
5.	Cyst with hemorrhagic fluid	1	1.2 %
	Total	84	100 %

Table no 4. states that Epithelial Ovarian neoplasm , most commonly presented as pure cystic masses (61 %).

Tumor Laterality

It was seen that 29 cases were from right ovary, 29 from left ovary and 21 in bilateral ovaries.

Tumor size was measured , which ranged from <5 cms in 15.5 % to >10 cms in 70 % cases.

Malignant serous neoplasms were graded according to the recent 2 tier system, of classification. High grade tumours exhibit marked nuclear atypia and >12 mitosis /10 Hpf. From the above table 10, 2/8 cases (25%) were low grade carcinomas and 6/8 cases (75%) were high grade carcinomas. High grade carcinomas constitute the commonest type in the malignant serous tumours. All the 3 cases of malignant mucinous carcinomas presented with expansile type of invasion with back to back glands arrangement with no stroma in between. One case of endometroid carcinoma presented were with low grade.

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Immunohistochemistry

The main focus of the study was expression of PD-L1. So, expression of PD-L1 was examined in the immunostained slides of epithelial ovarian neoplasm. Firstly, area having fibrosis, adipose tissue or necrosis were avoided during examination. The control came positive. The intensity and extent of membranous staining by anti – PD L-1 were examined. Out of the 84 cases none of the cases showed intense positivity, fair positivity and weak or focal positivity. Overall, 100 % cases showed PD-L1 negative expression.

II. DISCUSSION

The present study is a cross sectional descriptive analysis of epithelial ovarian carcinoma that were submitted to Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi for histopathological examination from January 2015 to August 2022 and were diagnosed as epithelial ovarian carcinoma . Immunohistochemical studies of PD- L1 were carried on 84 neoplastic and borderline cases.

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 [(7)]. 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 [(8)]. 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 [(9).]

A wide age range is noted in the present study with the youngest patient at 17 years of age and the eldest at 81 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 35 to 55 years of age. Out of the 84 patients, 9 were nulliparous, and the remaining 75 cases were multiparous. Majority of the tumours are unilateral (34%) which is slightly higher than was observed by Shirish et al [(10)], with a little predilection towards the right side tumour (34%) than the left side (32%). The incidence of bilateral tumours is 25% similar to a earlier study [(11).]

The size of the tumours was compared with other clinicopathological parameters. In majority of the patient the tumours were more than 10 cm in size (70 % cases) [(11).]

In our study, expression of PD- L1 has been evaluated in tumour 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 our study, the expression of PD-L1 in all the tumours came negative. This may be because of the limited study population and selection bias. Overall, No expression of PD-L1 in tumour cells is seen in 84 cases. Similar incidence has been seen in many study like Kahraman et al[(12)]where no expression of PD-L1was seen in tumour cells, Dennis et al[(13)]with only 3.5% cases showing high expression of PD-L1 in tumour cells. One Indian study was also done on PD- L1 and has showed high expression only in 6 % cases, 81 % cases with no expression of PD-L1 in tumour cell.[(14)]

III. SUMMARY AND CONCLUSION

Above study concluded that :

The ratio of benign and malignant ovarian neoplasm is 3:1.,with unilateral tumours (68%) being more common.

Most common gross presentation of malignant tumors was purely cystic in 60 % cases.

Regarding Histological type, the most common neoplasm among was malignant serous tumour (40 %) and . none of the tumor showed the expression of PD-LI.

Current study was an institution based study, with a small sample size of 84 cases. The results may not actually reflect the original age distribution and histological pattern of ovarian tumours in Indian population. The epidemiological data of developed countries in many aspects differ from the developing nations. The differences about hormone receptors expression by different authors may be due to various parameters like case selection.

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Microphotograph :

Fig 1 :Placenta as control

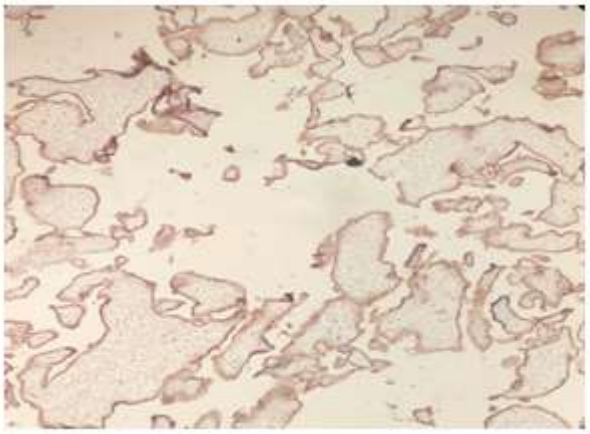

Fig no 1 : control positivity of PD-L1 : Outer surface of syncytiotrophoblast was stained positive. Cytotrophoblasts were negatively stained (IHC, 400X)

Fig 2 : Malignant ovarian Serous carcinoma :

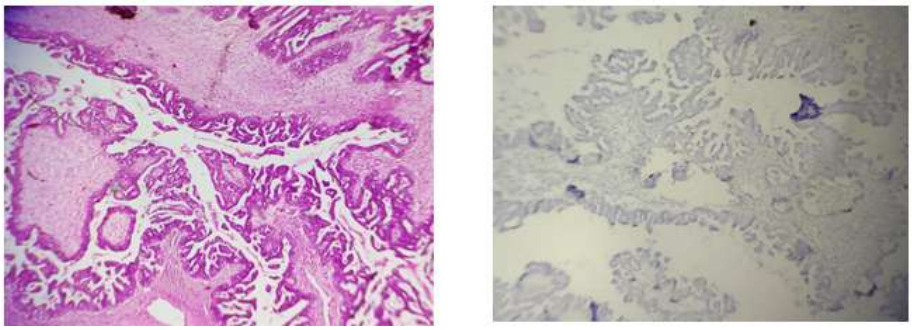

Fig 2 shows H & E stain 10 x view showing papillary arrangement and marked degree of cytological atypia and pleomorphism and PD-L1 IHC stain shows negative membranous staining

Fig no 3 :Mucinous ca

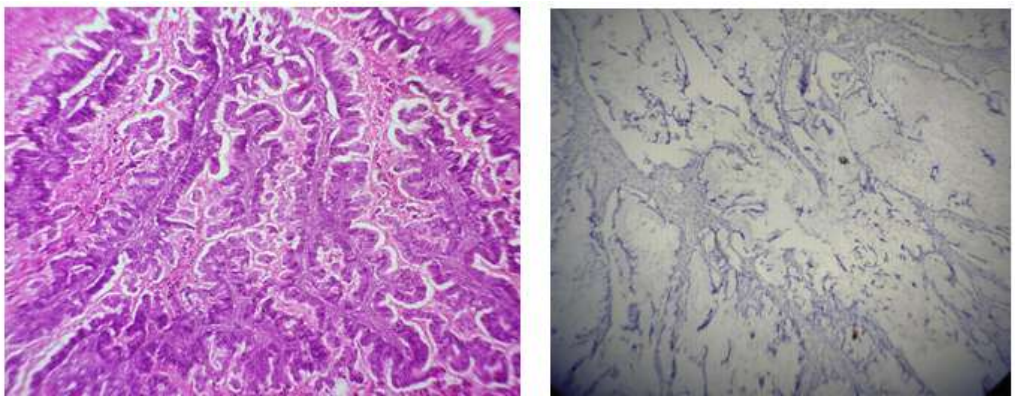

Fig no3 : shows mucinous ovarian carcinoma showing marked glandular crowding and very little intervening stroma. The lumen of the glands are filled with, mucin. Glands are lined by malignant mucinous epithelium showing marked nuclear atypia and intracytoplasmic mucin ((H and E, 10), and PD-L1 IHC was found to be negative for membranous staining.

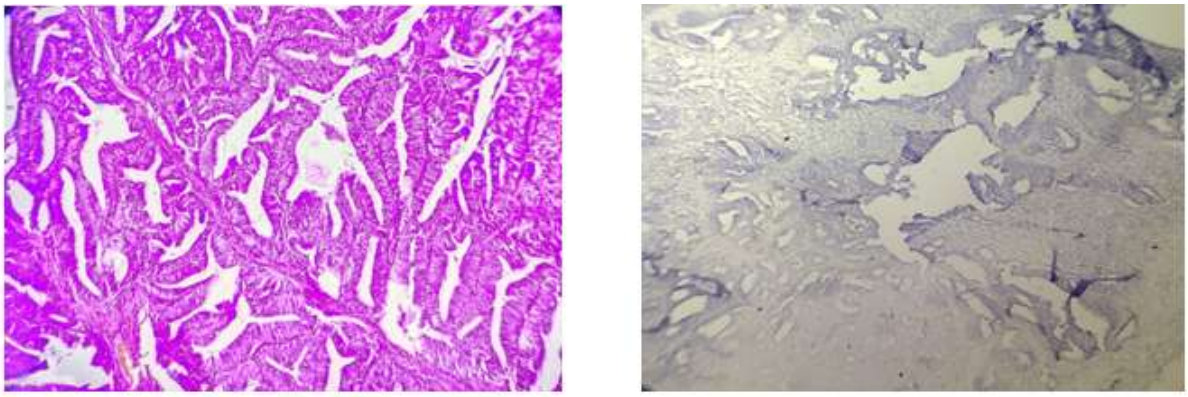

Fig no 4.Ovarian Endometrioid Carcinoma

Fig 4 : shows Endometroid carcinoma showing back to back arrangement of the glands with stromal invasion, cells are lined by columnar cells with atypia. And PD-L1 IHC shows negative membranous staining.

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