Original Article

Expression of Programmed Cell Death Ligand-1 and Mismatch Repair Status in Endometrial Carcinomas

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Abstract

Background and Aims: Programmed death ligand-1 (PD-L1) is a co-regulatory molecule that suppresses local immunity, and mismatch repair (MMR) deficiency (dMMR) is reported to influence the response to anti-PD-L1-targeted therapy. This study was conducted to find the PD-L1 status, the occurrence of dMMR in endometrial carcinomas, and the association between them. **Materials and Methods:** The study included 35 resected specimens of endometrial carcinomas represented on formalin-fixed paraffin-embedded sections from January 2016 to July 2020. The clinicopathologic information including patient age, tumor histologic type, grade, stage, lymphovascular invasion, the extent of myometrial invasion, and the percentage of tumor-infiltrating lymphocytes (TILs) were obtained in all cases. The expression of PD-L1 and MMR antibodies including mutS homolog 2 (MSH-2), MSH-6, mutL homolog 1 (MLH-1) and MLH-3, and postmeiotic segregation 2 were assessed using immunohistochemistry. The statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) version 26. **Results:** PD-L1 expression was noted in 48.6% of the cases in tumor cells and 65.7% of the cases in TILs and MMR was deficient in 28.6% of endometrial carcinomas. A statistically significant relation was noted between dMMR and TILs, PD-L1 expression in tumor cells and TILs, PD-L1 expression in tumor cells, and extent of myometrial invasion. Although there was no statistically significant association between MMR status and PD-L1 expression in tumor cells or TILs, 60% of patients with dMMR were PD-L1 positive. **Conclusion:** Sixty percent of dMMR cases showed PD-L1 expression in tumor cells. We conclude, ECs that are MMR deficient might get better response to anti-PD-L1 therapy. This study also revealed the prognostic use of TILs in PD-L1-expressed tumors.

Keywords: *B7-H1 antigen, DNA mismatch repair, immune evasion, pembrolizumab, tumor-infiltrating lymphocytes*

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Introduction

Endometrial carcinoma is the sixth most common gynecologic malignancy worldwide and 15th most common in India.[1] Early stages are often treated with surgery alone, while the advanced stage tumors have a poor prognosis requiring radiotherapy with or without chemotherapy and hormonal therapy in addition to surgery. Recently, immunotherapy has been used as a second-line treatment option for advanced and refractory endometrial carcinoma cases. The last decade has witnessed remarkable progress in the development of checkpoint blockade immunotherapy, particularly drugs that target programmed cell death-1 (PD-1) and PD-1 ligand-1 (PD-L1). PD-L1 expressed on tumor

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cells binds to PD-1 receptors on activated T-cells, inhibiting cytotoxic T-cells. Thus, it helps in the evasion of immune surveillance. The expression of PD-L1 by tumor cells and infiltrating immune cells has been verified by immunohistochemistry (IHC) in a variety of tumors, suggesting a role for the PD-1/PD-L1 axis as a prognostic trait and therapeutic target across multiple histotypes of endometrial carcinomas.[2] Currently, there are three approved PD-L1 inhibitors by the U.S. Food and Drug Administration for cancer treatment, including pembrolizumab, atezolizumab, and nivolumab.[3] The mismatch repair (MMR) pathway is a single-strand break repair mechanism for DNA replication errors. As MMR substantiates genomic integrity and stability, its failure results in microsatellite instability (MSI). The MMR deficiency (dMMR) status has been widely detected in Lynch syndrome or hereditary nonpolyposis colon cancer, which includes colorectal carcinoma, gastrointestinal adenocarcinoma, and endometrial carcinoma.[4] However, dMMR is sporadically identified in endometrial carcinoma, ovarian carcinoma, gastric adenocarcinoma, colorectal carcinoma, and melanoma. MMR defects are caused by mutations or epigenetic silencing in mutL homolog 1 (MLH-1), mutS homolog 2 (MSH-2), MSH-6, MLH-3, postmeiotic segregation 1 (PMS-1), or PMS-2. If one or more proteins are not expressed or dysfunctional, it is termed dMMR. Laboratory studies are available to evaluate the status of the MMR pathway, including IHC staining for MMR proteins, polymerase chain reaction (PCR) to evaluate MSI, MLH-1 promoter methylation analysis, and *BRAF* sequencing. These tests are performed directly on tumor samples having prognostic and therapeutic implications.[5,6] Tumors with dMMR are sensitive to immune checkpoint blockade, particularly PD-1 and PD-L1 inhibitors. MMR status could also serve as a candidate biomarker and predict the responses to therapy with anti-PD-1 and anti-PD-L1. This study was designed to evaluate the expression and interplay of PD-L1 and MMR status in endometrial carcinomas and to assess the association between PD-L1 and MMR status.

Materials and Methods

This study was done on resected specimens of endometrial carcinomas in the Department of Pathology at AIIMS, Jodhpur, from January 2016 to July 2020. Approval from the Institutional Review Board was obtained at the initiation of the study (IEC number: AIIMS/IEC/2018/759). Based on the convenience sampling method, 35 cases of endometrial carcinomas were included, and tumors were represented on formalin-fixed paraffin-embedded. Clinicopathologic information, including patient age, tumor histologic type, grade, stage, lymphovascular invasion, the extent of myometrial invasion, and tumor-infiltrating lymphocytes (TILs), was obtained in all cases.

Immunohistochemistry

The diagnosis was reviewed on whole-tissue sections of hematoxylin- and eosin-stained slides. The percentage of TILs to viable tumor areas was assessed. Two pathologists selected representative sections, and the tumor tissues were fixed with formalin, embedded in paraffin, and cut into 4 μm-thick sections for IHC staining.

Programmed cell death-1 ligand

Immunohistochemical staining for PD-L1/CD274 (Prediluted, Clone: CAL10, Company: Biocare Medical) was performed on all cases manually. PD-L1 immunohistochemical staining was interpreted as positive when clear membranous staining was present in ≥1% of tumor cells and membranous or cytoplasmic staining in TILs. A proportion score was given to all the cases based on the extent of staining: 1% to 5%, 6% to 10%, 11% to 25%, 26% to 50%, and >50% [Figure 1]. The combined positive score (CPS) is the ratio of the number of PD-L1-expressing cells, including tumor cells, lymphocytes, and macrophages, to the number of all tumor cells.[7,8] CPS can evaluate PD-L1 expression in the tumor microenvironment, reflecting the host immune status and malignant tumor potential.

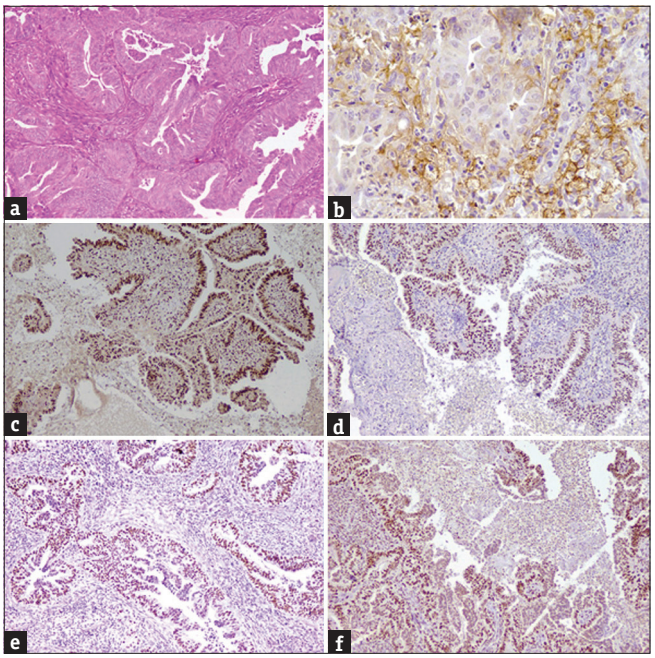

Figure 1: A case of low-grade endometrioid carcinoma (a) with programmed death ligand-1 expression in tumor cells and tumor-infiltrating lymphocytes (b). The tumor is mismatch repair stable and shows retained MSH-2 protein (c), retained MSH-6 protein (d), retained MLH-1 protein (e), and retained postmeiotic segregation-2 protein (f). MSH: MutS homolog, MLH-1: MutL homolog-1

CPS scoring was performed, and a score of ≥1 was considered positive.

Mismatch repair protein status

MSH-2 (Preparation: Prediluted, Clone: FE11, Company: Dako), MSH-6 (Preparation: Prediluted, Clone: EP49, Company: Dako), MLH-1 (Preparation: Prediluted, Clone: ES05, Company: Daco) and PMS-2 (Preparation: Prediluted, Clone: A 16-4, Company: Biocare Medical) proteins were included. Any nuclear staining within the tumor cells is considered positive, and the complete absence of nuclear staining within the tumor cells with concurrent positive internal control was considered negative. The cases were interpreted as MMR retained when all the four antibodies showed positive nuclear staining of the tumor cells and dMMR when one or more antibodies showed an absence of nuclear staining of the tumor cells.

Statistical analysis

Qualitative data such as PD-L1 and dMMR were expressed as percentages. Data were analyzed by IBM-SPSS 26.0 version. Inferential statistics were done by using Fisher's exact test and the Chi-square test.

Results

In the study of 35 cases of endometrial carcinomas, 29 cases were more than 50 years of age and the youngest patient was 25 years old. Most cases were low-grade tumors (82.9%) and belonged to Stage I (74.3%). Myometrial invasion of >50% was observed in 77.1% of the cases [Table 1].

Ten out of 35 cases (28.8%) showed dMMR. Out of 10 cases who were dMMR, 6 cases (60%) expressed PD-L1 in tumor cells, and 9 cases (90%) had a CPS of ≥1 [Table 2]. A CPS score of ≥1 was observed in 28 cases of EC (80%). The combined loss of PMS-2 and MLH-1 protein was noted in 7 cases (20%). The combined loss of MSH-2 and MSH-6 was noted in 3 cases (8.6%). All the cases with dMMR had TILs of more than 5%. There was a statistically significant association between dMMR and more TILs [Table 2].

Among 35 cases of EC, PD-L1 expression in tumor cells was present in 17 cases (48.6%). None of the cases had extensive (>50%) PD-L1 staining. The PD-L1 expression in tumor cells was present in 87.5% of cases, with myometrial invasion <50% (P = 0.018). The majority of ECs (65.7%) showed PD-L1 expression in TILs. Out of the 17 cases who expressed PD-L1 in tumor cells, 14 cases (82.3%) showed PD-L1 expression in TILs [Table 3]. Figures 2 and 3 show the PD-L1 expression in TILs and tumor cells, and dMMR.

Table 1: Clinical and pathological parameters (*n*=35)

Features	Characteristics	Frequency, *n* (%)
Age (years)	≤50	6 (17.1)
	>50	29 (82.9)
Grade	I	25 (71.4)
	II	4 (11.4)
	III	6 (17.1)
Stage	I	26 (74.3)
	II	1 (2.9)
	III	7 (20.0)
	IV	1 (2.9)
Histological diagnosis	Endometrioid	35 (100)
Myometrial invasion (%)	≤50	8 (22.9)
	>50	27 (77.1)
Lymphovascular invasion	Absent	26 (74.3)
	Present	9 (25.7)
TILs	Absent	2 (5.7)
	Present	33 (94.3)

TILs: Tumor-infiltrating lymphocytes

Table 2: Mismatched repair status and programmed cell death ligand-1 expression in tumor cell, combined positive score, and tumor-infiltrating lymphocyte percentage

Features	Characteristics	MMR status		*P*
		Deficient (*n*=10), *n* (%)	Retained (*n*=25), *n* (%)	
PD-L1 expression in tumor cells	Absent (*n*=18)	4 (40)	14 (56)	0.471
	Present (*n*=17)	6 (60)	11 (44)	
CPS	<1	1 (10)	6 (24)	0.644
	≥1	9 (90)	19 (76)	
TILs	<5	0	4 (16)	0.034
	5–10	1 (10.0)	9 (36)	
	11–50	7 (70)	12 (48)	
	≥50	2 (20)	0	

PD-L1: Programmed cell death ligand-1, CPS: Combined positive score, TILs: Tumor-infiltrating lymphocytes, MMR: Mismatched repair

Table 3: Programmed cell death ligand expression in tumor cells and programmed cell death ligand expression in tumor-infiltrating lymphocytes

PD-L1 expression in TILs	PD-L1 expression in tumor cells, total number of cases=35		*P*
	Absent (*n*=18), *n* (%)	Present (*n*=17), *n* (%)	
Absent (*n*=12)	9 (50)	3 (17.6)	0.075
Present (*n*=23)	9 (50)	14 (82.3)	

PD-L1: Programmed cell death ligand-1, TILs: Tumor-infiltrating lymphocytes

Discussion

Advanced stages of EC have poor prognosis and are refractory to surgical treatment. It possesses more immune cells such as lymphocytes, macrophages, and

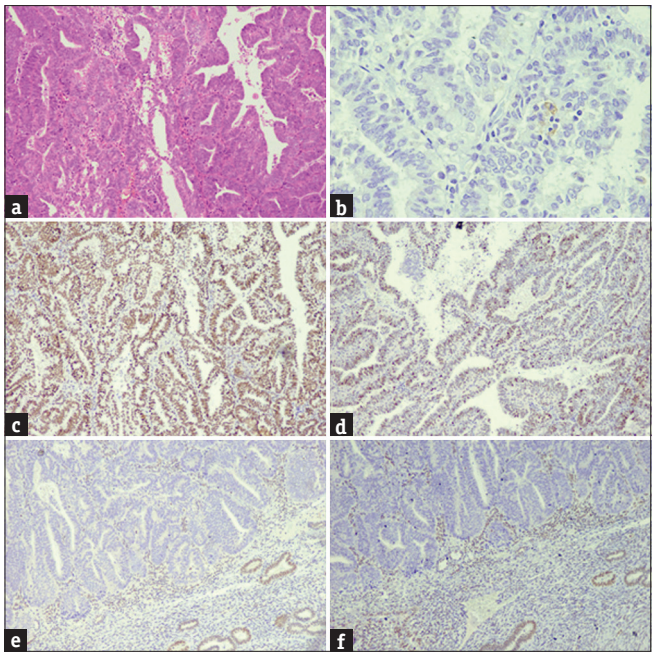

Figure 2: A case of low-grade endometrioid carcinoma (a) with PD-LI expression in 2% of tumor-infiltrating lymphocytes and absence of PD-L1 expression in tumor cells (b). The tumor is mismatch repair deficient and shows retained MSH-2 protein (c) and retained MSH-6 protein (d), with a combined loss of MLH-1 protein (e) and postmeiotic segregation-2 protein (f). PD-LI: Programmed death ligand-1, MSH: MutS homolog, MLH-1: MutL homolog-1

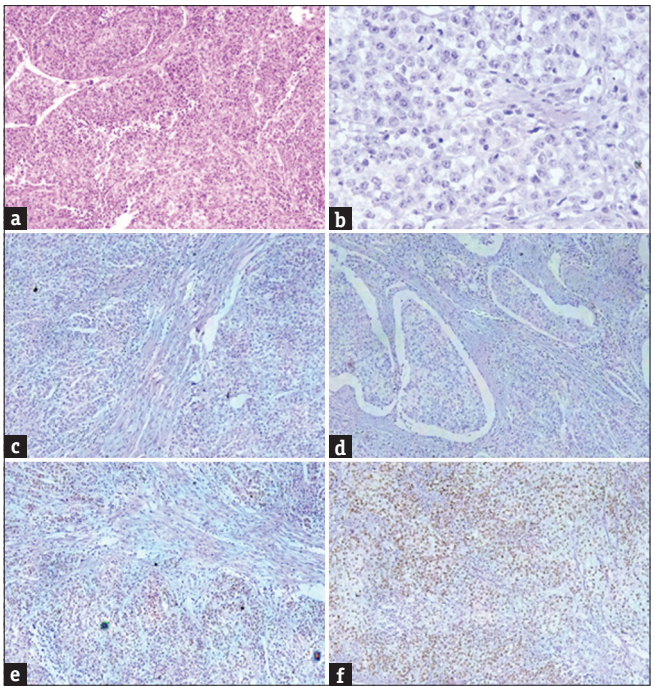

Figure 3: A case of high-grade endometrioid carcinoma (a) with the absence of programmed death ligand-1 expression in tumor cells and tumor-infiltrating lymphocytes (b). The tumor is mismatch repair deficient and shows combined loss of MSH-2 protein (c) and MSH-6 protein (d), with retained MLH-1 protein (e) and retained postmeiotic segregation-2 protein (f). MSH: MutS homolog, MLH-1: MutL homolog-1

dendritic cells, which contain PD-1 receptors. PD-L1 expressed on tumor cells binds to PD-1 receptors on activated T-cells, inhibiting cytotoxic T-cells. Thus, it helps in the evasion of immune surveillance. By understanding the PD-1/PD-L1 axis, it is hypothesized that immunotherapy can improve the prognosis of advanced-stage tumors.[9] The dMMR tumors have been associated with increased TILs and high neoantigen production. Additionally, due to the increased expression of PD-L1 in TILs of dMMR tumors, it is suggested that anti-PD-L1 therapy is more helpful in dMMR cases.[10,11]

TILs are essential to the tumor microenvironment and reflect the host antitumor immune response.[12,13] The presence of TILs signifies a better prognosis in endometrial carcinomas, breast carcinomas, ovarian malignancies, and colorectal carcinomas.[14] The present study observed TILs in 33 out of 35 cases. Tumors categorized as higher grade, higher stage, and with LVI had fewer TILs. We found PD-L1 to be expressed in 48.6% of EC cases. Studies on PD-L1 expression in tumor cells were variable, ranging from 15% to 83%.[15-17] The variations in the percentage of expression might be due to differences in the clone of PD-L1 used, tumor heterogeneity, and inherent genetic and environmental differences among the Indian population.

There was a statistically significant association between PD-L1 expression in the tumor cells and the extent of myometrial invasion (P = 0.018) and advanced-stage disease (P = 0.039). It was observed that the tumors expressing PD-L1 were more often associated with >50% of myometrial invasion. Most studies have concluded that PD-L1 expression in tumor cells was associated with adverse prognostic factors such as an advanced stage, high histologic grade, and deep myometrial invasion.[18-22] The recent approval of a CPS, which calculates PD-L1 staining in both tumoral and TILs, suggests that focal immune cell expression of inhibitory checkpoint molecules may be sufficient to warrant targeted treatment.[23-26] The CPS is a robust and reproducible scoring system for assessing the predictive response of PD-L1 immunotherapy.[18,27] Currently, it is successfully used in gastroesophageal carcinomas, head-and-neck squamous cell carcinoma, and non-small cell lung carcinoma. In this study, we used CPS in EC to quantify the expression of PD-L1 in tumor cells and TILs. Expression of PD-L9961 in TILs limits the antitumor activity of cytotoxic T-cells and promotes tumor progression.[28,29] In the present study, PD-L1 expression in TILs was observed in 65.7% of cases of ECs, and CPS was ≥1 in 80% (28 cases) of cases, indicating that a large proportion of cases could be benefited from immunotherapy.

Mismatch repair status

The importance of MMR status has been emphasized in the recent molecular classification of EC, with four distinct molecular subgroups. The cancer genome atlas (TCGA) project classified ECs into four groups – POLE ultramutated (POLE EDM), MSI hypermutated (MMR deficient, MSI), copy number (CN) high (p53 mutation), and CN low (NSMP – no specific molecular profile). Patients with POLE mutation and MSI subgroups showed better survival outcomes than the p53 mutant group and the NSMP group.[30] The research of dMMR allows screening of patients with endometrial carcinomas to segregate Lynch syndrome from sporadic endometrial cancers, which have specific prognostic and therapeutic implications, mainly targeted therapies.[31] In the present study, 28.6% of cases showed loss of one or more MMR proteins. This finding concord with TCGA data, which says that up to 30% of EC patients have MMRd tumors.[32] Nine out of ten dMMR cases showed >50% myometrial invasion representing an adverse prognosis of dMMR cases in EC. The dMMR cases showed a higher percentage of TILs ($P = 0.034$) in our study, depicting a favorable feature for the implementation of immunotherapy.[33-35] Literature suggests that dMMR tumors possess more immune checkpoint ligands PD-1, PD-L1, and CTLA-4 that resist tumor elimination.[36]

In the present study, 80% of dMMR cases showed PD-L1 expression in TILs, and 60% of dMMR cases showed PD-L1 expression in tumor cells. Subsequently, the same concepts form the basis of anti-PDL1 therapy in MMR-deficient EC. As studies in the Indian population on MMR status and PD-L1 expression in ECs are limited, this study will make a new scope for further research on the association of dMMR and PD-L1 expression on tumor cells in endometrial carcinoma. The novel study may aid in the development of new diagnostic modalities and targeted therapy for endometrial carcinoma through further research. The strengths of the study are as follows: in advanced cases of endometrial carcinoma that are refractory to surgery, radiotherapy, and chemotherapy, targeted immunotherapy is an emerging treatment modality. This study adds insight to the existing literature on immunotherapy. In addition to the primary outcome, the association of PD-L1 and MMR expression with clinicopathological parameters, including TILs in endometrial carcinoma, was also evaluated. The CPS scoring system, which is a robust method of scoring PD-L1 staining pattern, was utilized in this study that could predict the response to pembrolizumab.

Limitation of this study

The results might be affected by a smaller sample size and single-center study. The isolated mutation of MMR genes could not be evaluated because of the inability to perform PCR and POLE testing. In further research methodology, PCR and POLE testing could be added to gain more insight into the molecular pathology of endometrial carcinoma. The results of this study cannot be extrapolated to other gynecological malignancies as it was performed only in endometrial carcinoma.

Conclusion

This study underscores the need to evaluate the MMR status in endometrial carcinomas as a routine practice, as it will provide valuable information for the management and prognostication of the patients. In our study population of EC, almost half of the endometrial carcinomas showed PD-L1 expression, and one-fourth to one-third of cases were MMR deficient. Sixty percent of dMMR cases showed PD-L1 expression in tumor cells. Hence, endometrial carcinomas that are dMMR could respond better to anti-PD-L1 therapy. While dMMR cases were associated with favorable prognostic factors like TILs, PD-L1-expressing endometrial carcinomas were associated with adverse prognostic factors such as increased myometrial invasion and reduced TILs. Additional studies on larger cohorts are required for the same to enable the selection of patients likely to benefit from targeted therapy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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