Original Article

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Expression of PD-L1 in Microsatellite Instability High Tumours: A Retrospective Study

Pathology Section

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ABSTRACT

Introduction: Microsatellite Instability (MSI) is the hallmark of Lynch syndrome/Constitutional Mismatch Repair Deficiency (CMMRD) and is also found in many sporadic cancers like colorectal cancer, endometrial, gastric, small intestine, urothelial, central nervous system and sebaceous gland neoplasms. MSI is a predictive biomarker for immunotherapy and Immunohistochemistry (IHC) antibodies against four Mismatch Repair (MMR) proteins: MutL Homolog 1 (MLH1), MutS Homolog 2 (MSH2), MutS Homolog 6 (MSH6) and Postmeiotic Segregation increased 2 (PMS2) can identify the MSI status of the tumour. In addition to MSI, immune checkpoint Programmed Cell Death Protein 1 (PD-1) expression and its ligand PD-L1 are biomarkers that can predict response to immunotherapy. Considering this increasing interest to identify deficient MMR (dMMR) status in different cancers, authors have assessed the expression of PD-L1 and status of MSI in various cancer types.

Aim: To evaluate the expression of PD-L1 in MSI-high status tumours.

Materials and Methods: This retrospective cross-sectional study was done in the Department of Pathology, Panimalar Medical College Hospital and Research Institute, Chennai, Tamil Nadu, India for a period of six months from July 2022 to December 2022. A total of 151 cases were identified for the period of three years from January 2020 to December 2022. The slides and blocks were retrieved from the archives. Tumour sections from the paraffin embedded tissues were deparaffinised and antigen retrieval was done. IHC using four antibodies (MLH1, MH2, MSH6 and PMS2) was performed on these slides to assess the MSI status. The slides were reviewed and were further subjected to PD-L1 IHC. PD-L1 expression on tumour cells was compared with the MSI status of different cancer types. The p-value was calculated using t-test and $p<0.5$ was considered statistically significant. Statistical analysis was done using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) software version 21.0.

Results: A total of 151 cases were included in the present study. A positive nuclear stain for the four MMR proteins denote expression of wild type MMR proteins, hence MSI low status. A loss of nuclear expression denotes mutation of MMR proteins and hence MSI high (MSI-H) status. The MSI was high in nine out of 48 cases (18.75%) of colon cancer, three out of 15 cases (20%) of endometrial cancers, three out of 20 cases (15%) of gastric cancers. Rest were one out of sixteen cases of ovarian cancer, one out of two malignant melanoma and one out of three glioblastoma. Out of these, PD-L1 was positive in seven of the 18 MSI-H cases (38.88% of MSI-H cases). The MSI was low/stable in the remaining 133 cases. The p-value of significance was 0.03 (statistically significant).

Conclusion: This study shows a significant association of MSI-H with PD-L1 expression in tumours. Further large scale studies can help in assessing the role of PD-L1 as an effective therapeutic biomarker in MSI-high status patients who can benefit from targeted therapy.

Keywords: Lynch syndrome, Mismatch repair deficiency, Mismatch repair proteins, Programmed cell death protein ligand 1

INTRODUCTION

The accumulation of dMMR mutations in monomorphic microsatellites as short tandem repeats which are prone to mismatch errors is termed as MSI. Although, MSI is the hallmark of Lynch syndrome and CMMRD, it has been found in many sporadic cancers belonging to this spectrum, such as gastric, small intestinal, colorectal, endometrial, urothelial, central nervous system and sebaceous gland neoplasms [1,2]. Various studies suggest that MSI is a predictive biomarker for immunotherapy [3,4]. MSI can be identified by the use of IHC and molecular tests like Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS). IHC uses antibodies against four MMR proteins: MLH1, MSH2, MSH6 and PMS2. The immune checkpoint PD-1 expression and its ligand PD-L1 are also biomarkers that can predict response to immunotherapy [5,6] and can be identified by the use of IHC on tumour sections. Considering this increase in the interest to identify dMMR status in different cancers, authors have assessed the expression of PD-L1 and status of MSI in various cancer types. This can provide valuable information to identify patients with MSI high status and PD-L1 expression, who may be potential candidates for targeted therapy. Therefore, the aim of the study was to assess the MSI status of different cancer types and to assess the distribution of PD-L1 expression of these cancers with the MSI status.

MATERIALS AND METHODS

This retrospective cross-sectional study was done in the Department of Pathology, Panimalar Medical College Hospital and Research Institute, Chennai, Tamil Nadu, India. for a period of six months from July 2022 to December 2022. A total of 151 cancer cases were identified for the period of three years from January 2020 to December 2022. The slides and blocks were retrieved from the archives.

Inclusion criteria: Biopsies and resection specimens of cancer cases with adequate tissue; blocks with adequate representation of tumour. Tumours of the gastrointestinal tract, lung, thyroid, breast, endometrium, ovary, cervix, malignant melanoma and glioblastoma were included in the present study.

Exclusion criteria: Inadequate tissue for sectioning; inadequate representation of tumour; cytoplasmic staining of MSI by tumour cells; positive PD-L1 expression by immune cells in normal mucosa,

ulcerated areas and adenoma; pale cytoplasmic staining of PD-L1 were excluded from the study.

Study Procedure

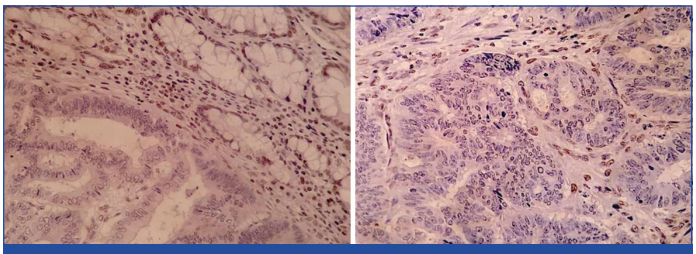
The IHC using four antibodies {Mouse monoclonal antibody MLH1 (Clone: GM011), Rabbit monoclonal antibody MSH2 (clone: RED2), Rabbit monoclonal antibody MSH6 (Clone: EP49) and Rabbit monoclonal antibody PMS2 (clone: EP51)} was performed on these slides to assess the MSI status. The slides were reviewed and were further subjected to PD-L1 IHC (Clone SP263). The tumour sections from paraffin embedded tissues were taken on polylysine coated slides, incubated overnight at 37°C and deparaffinised. After antigen retrieval in Tris-EDTA buffer, blocking was done with 3% hydrogen peroxide. Primary antibody was added followed by target binder and Horseradish peroxidase. The slides are then treated with Diaminobenzidine (DAB) chromogen, stained with haematoxylin and mounted with DPX (Dibutylphthalate Polystyrene Xylene). A positive nuclear stain for the four MMR proteins denote expression of wild type MMR proteins, hence MSI low status. A loss of nuclear expression denotes mutation of MMR proteins and hence MSI high (MSI-H) status. The percentage of PD-L1 positive tumour cells out of total tumour cells was estimated. Membranous staining of >1% of the tumour cells was considered as positive. CPS was calculated by dividing the total number of PD-L1 positive cells by the total number of viable tumour cells. Score >1% was considered to be positive [7].

STATISTICAL ANALYSIS

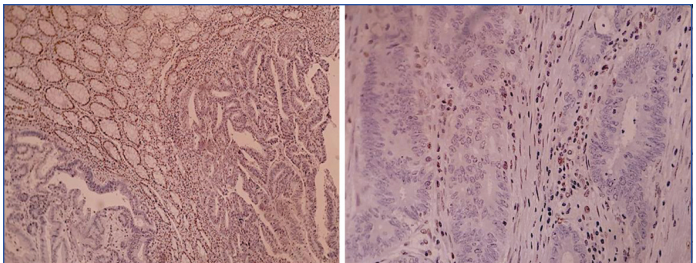
The PD-L1 expression on tumour cells was compared with the MSI status of different cancer types. Statistical analysis was done using IBM SPSS software version 21.0. The p-value was calculated using t-test and p <0.5 was considered statistically significant.

RESULTS

A total of 151 cases were included in the present study, out of which 87 (57.6%) were males and 64 (42.4%) were females. The mean age was 53.5 years (26-81 years). MSI-H was observed in 18 cases (11.92% of cases). The mean age was 66.5 years (42-91 years), out of which 11 were males and seven were females. The MSI was high in nine out of 48 cases (18.75%) of colon cancer [Table/Fig-1,2], three out of 15 cases (20%) of endometrial cancers, three out of 20 cases (15%) of gastric cancers [Table/Fig-3,4].

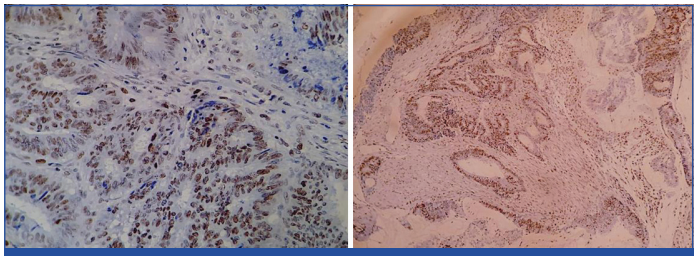


[Table/Fig-1]: Loss of nuclear expression of MLH1 by tumour cells. (Normal glands show retained nuclear expression) (400x magnification)
[Table/Fig-2]: Loss of MSH2 by tumour cells (400x magnification). (Images from left to right)

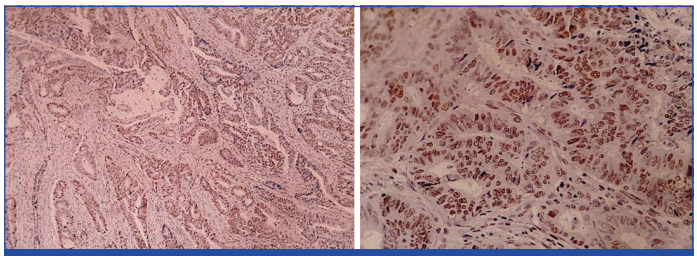


[Table/Fig-3]: Loss of MSH6 in tumour cells. (40x magnification) (Normal glands (upper left) show retained nuclear expression)
[Table/Fig-4]: Loss of PMS2 in tumour cells (400x magnification). (Images from left to right)

Rest includes one out of sixteen cases of ovarian cancer, one out of two malignant melanomas and one out of three glioblastoma. The MSI was low in 133 cases [Table/Fig-5-8]. PD-L1 was positive in seven of the 18 MSI-H cases (38.88% of MSI-H cases), which included five cases of colon cancer, one gastric cancer and one endometrial cancer. The p-value of significance was 0.03 (statistically significant).

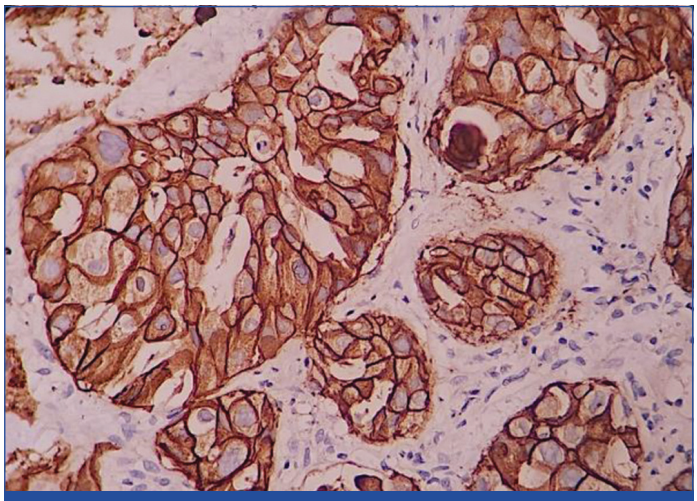


[Table/Fig-5]: Retained nuclear expression of MLH1 by tumour cells (400x magnification).
[Table/Fig-6]: Retained nuclear expression of MSH2 (40x magnification). (Images from left to right)



[Table/Fig-7]: Retained MSH6 by tumour cells (40x magnification).
[Table/Fig-8]: Retained PMS2 nuclear expression (400x magnification). (Images from left to right)

Among the 18 MSI-H cases, 12 were moderately differentiated adenocarcinoma, four were poorly differentiated adenocarcinoma, one malignant melanoma and one glioblastoma. PD-L1 positivity was seen in 11 cases- one squamous cell carcinoma (lung), one neuroendocrine tumour (gastric), five poorly differentiated adenocarcinoma (one gastric, two colon, one lung and one endometrium), one Grade-2 infiltrating ductal carcinoma breast [Table/Fig-9] and three were moderately differentiated adenocarcinoma (colon).



[Table/Fig-9]: PD-L1 expression by tumour cells (400x magnification).

The MSI was low/stable in the remaining 133 cases. The mean age was 62 years (26-98 years). Among these, 79 were males and 54 were females. PD-L1 was positive in 4 cases (3% of MSI low/stable cases), including two cases of lung cancer, one case each of gastric and breast cancer. The distribution of MSI status and PD-L1 among different cancers is summarised in [Table/Fig-10]. The tumour cell score of PD-L1 was >10% in five out of seven cases of MSI-H. Rest of the cases had tumour cell score of <10%. The CPS was >10% in two cases of MSI-H. The CPS was positive in one MSI low/stable case and it was <10%.

S. No.	Site	Total no. of cases	High			Low		
			MSI-H	PD-L1	CPS >10%	MSI Low/ Stable	PD-L1	CPS >10%
1	Oesophagus	5	0	0	0	5	0	0
2	Stomach	20	3 (15%)	1	0	17	1	0
3	Small intestine	3	0	0	0	3	0	0
4	Colon	48	9 (18.75%)	5	2	39	0	0
5	Lung	8	0	0	0	8	2	0
6	Breast	24	0	0	0	24	1	0
7	Endometrium	15	3 (20%)	1	0	12	0	0
8	Ovary	16	1 (6.25%)	0	0	15	0	0
9	Cervix	3	0	0	0	3	0	0
10	Malignant melanoma	2	1 (50%)	0	0	1	0	0
11	Glioblastoma	3	1 (33.33%)	0	0	2	0	0
12	Thyroid	4	0	0	0	4	0	0
	Total	151	18	7	2	133	4	0

[Table/Fig-10]: Distribution of MSI status and PD-L1 among different cancers.

DISCUSSION

Microsatellites are short tandem repeats composed of repeating DNA sequences and found throughout the genome, in both the coding and non coding regions. They are highly polymorphic in different individuals but are of the same length in germline DNA and somatic DNA of tumour in the patient. Microsatellites are highly susceptible to DNA mismatch errors during the DNA replication process or any iatrogenic damage [8]. These errors are usually rectified by the DNA MMR mechanism. The four critical genes involved in this process are MLH1, MSH2, MSH6 and PMS2. MSI is a defect in these DNA MMR genes which results in genetic hypermutability. dMMR occurs when any of these genes are inactivated either by germline and/or somatic mutations or epigenetic silencing [9]. Mutations resulting in defective MSH6 can be compensated in the heterodimer by MSH3, and PMS2 by PMS1 or MLH3. MLH1 and MSH2 gene mutations cause proteolytic degradation of PMS2 and MSH6, whereas PMS2 or MSH6 mutations may not result in proteolytic degradation MLH1 or MSH2. A tumour with large number of clustered mutations in microsatellites and harbouring MSI is called a dMMR tumour. Hence, MSI is a marker of dMMR. IHC or PCR tests can be used to recognise dMMR/MSI in sporadic cancers which belong to the CMMRD spectrum like gastric, colorectal, small intestinal, endometrial, urothelial, central nervous system and sebaceous gland tumours [10-12]. There is no sufficient data available for cancers which are not part of this spectrum.

The IHC uses antibodies against the four MMR proteins: MLH1, MSH2, MSH6 and PMS2 for predicting the MSI status of patients. The MMR proteins are expressed in the cell nuclei. As explained earlier, MLH1 mutations result in IHC loss of MLH1 as well as PMS2, while MSH2 mutation shows IHC loss of MSH2 and also MSH6. Therefore, PMS2, IHC can identify cases harbouring MLH1 or PMS2 defects, while MSH6 can identify cases with MSH2 or MSH6 defects. But, standalone MLH1 and MSH2 IHC cannot recognise cases with PMS2 or MSH6 defects [13,14]. Preanalytical errors such as inadequate tissue fixation may result in false negative staining or aberrant patterns like cytoplasmic, dot-like or perinuclear staining. Hence, it is mandatory to include an internal positive control like normal mucosa [15]. Positive immunostaining in the presence of MMR deficiency can occur due to catalytically inactive but antigenic intact missense mutant MMR proteins or lack of PMS2 or MSH6 substituted by MLH3/PMS1 or MSH3 respectively. Therefore, it is recommended to use all the four IHC antibodies and whenever there is a doubt in the IHC, MSI-PCR should be done for confirmation [16]. Indeterminate IHC results such as loss of only one heterodimer subunit also warrants confirmation by MSI-PCR. MSI is loss of stability in ≥2 of the microsatellite markers. MSI can also be assessed by Next Generation Sequencing (NGS) [17]. The host antitumour immune function is negatively regulated by immune checkpoints which are critical for the suppression of the host antitumour immune reactivity. These immune checkpoints are the targets of tumour's ability to escape immunosurveillance. This forms the basis for the targeted treatment of human cancers using immune checkpoint inhibitors. T and B lymphocytes, Natural Killer (NK) cells and Tumour-Infiltrating Lymphocytes (TILs) express an inhibitory co-receptor PD-1 [18]. In the Tumour Microenvironment (TIME), PD-1 binds with the PD-L1 and inactivates the TILs resulting in immune resistance of the tumour [19]. The PD-1/PD-L1 pathway is a major negative modulator of immune response. Inhibition of this pathway by administration of monoclonal antibodies (mAbs) reactivating the Cytotoxic T Lymphocytes (CTLs) can be used to treat human cancers such as melanoma [20], renal cell carcinoma [21], and Non Small Cell Lung Cancer (NSCLC) [22].

Expression of PD-L1 by tumour cells is being used as a biomarker for targeted immunotherapy, while dMMR helps in predicting the response of tumours to PD-1 blockade [4,23]. There are reports of various clinical studies showing relatively more "sensitive" response of dMMR/MSI-H colorectal carcinoma patients to anti-PD-1/PD-L1 mAbs therapy when compared to proficient MMR (pMMR)/MSI-L cases [24,25]. The immune cell positivity for PD-L1 staining was found to be significantly higher in dMMR tumours than pMMR tumours [26]. The expression of PD-L1 on tumour cells has been found to be independent of MSI and EBV in cases of gastric carcinoma with lymphoid stroma [27]. There is significant correlation between high TMB and PD-L1 status in melanoma [28]; MSI cases are very rare in NSCLC, but the percentage of PD-L1 positive cases is very high, although it has been demonstrated that PD-L1 and TMB-high are independent in such cases. MSI-H/dMMR has been found in 1.16% lung cancer patients, most of which are Squamous Cell Carcinoma (SCC) [29]. MSI-H/dMMR is of limited prognostic value in triple negative breast cancer as their incidence is extremely low [30]. Studies are available which show higher PD-L1 expression in aggressive thyroid cancers suggesting that targeted therapy can help these cases [31]. Clinicians can predict response to anti PD-L1 therapy in their patients by evaluating the MSI and PD-L1 expression in the tumour and thereby select suitable patients for this therapy. This helps in identifying candidates who can benefit from anti-PD-1/PD-L1 therapy [32].

Limitation(s)

This study shows a significant association of MSI-H with PD-L1 expression in tumours. The limitations of the present study are the limited number of MSI-high cases available for comparison with PD-L1 and lack of confirmation of MSI status with PCR or NGS studies. Larger scale studies will help to establish a more definitive correlation between MSI status and PD-L1 expression in a variety of tumours.

CONCLUSION(S)

This study shows a significant association of MSI-H with PD-L1 expression in tumours. This can provide valuable information to identify patients with MSI-high status and PD-L1 expression, who may be potential candidates for targeted therapy. However, more extensive and large scale studies are required to standardise and recommend definitive cut-off values for PD-1/PD-L1 expression and also to study the response of tumours harbouring MSI to immune checkpoint blockade therapy. This can be a big breakthrough for cancer treatment worldwide.

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