Original Article

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Microsatellite Instability Status and Programmed Death Cell Ligand 1 Expression in Serous Ovarian Tumors

Seröz Over Tümörlerinde Mikrosatellit İnstabilite Durumu ve Programlanmış Ölüm- Ligand 1 Ekspresyonu

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ABSTRACT

Objective: The goal is to find out more about the microsatellite instability (MSI) and programmed death cell ligand 1 (PD-L1) expression in ovarian serous tumors so that we can better understand the tumor microenvironment and possibly come up with new ways to treat it. This study examines how PD-L1 expression and DNA mismatch repair (dMMR) are related in ovarian serous tumors. The goal is to figure out how these two factors affect the immune system of the tumor.

Methods: We used immunohistochemistry to examine MMR proteins and PD-L1 in 37 people with serous ovarian tumors. Of these, 14 had high-grade serous carcinoma, 6 had low-grade serous carcinoma, 8 had borderline serous tumors, and 9 had serous cystadenomas.

Results: In our study, only four cases (10.8%) showed loss of MMR protein expression, indicative of dMMR. We found no significant relationship between MSI status and tumor size, ovarian localization, International Federation of Gynecology and Obstetrics stage, or PD-L1 expression.

Conclusion: In this study, loss of MMR protein expression was not associated with prognostic parameters or PD-L1 expression. Although some studies have compared the MSI status of tumors with various prognostic parameters, a consensus has yet to be reached. Understanding the interplay between MSI and PD-L1 expression could guide personalized treatment approaches, offering new avenues for precision medicine in ovarian cancer.

Keywords: Microsatellite instability, PD-L1, serous ovarian cancer, immunohistochemistry

ÖZ

Amaç: Seröz over tümörlerinde mikrosatellit instabilitesi (MSI) ve programlanmış ölüm- ligand 1 (PD-L1) ekspresyonu hakkında daha fazla bilgi edinmek, böylece tümör mikroçevresini daha iyi anlayabilmek ve tedavi etmek için olası yeni yollar bulmaktır. Bu çalışmada seröz over tümörlerinde PD-L1 ekspresyonu ve DNA uyumsuzluk onarımının (dMMR) ilişkisi incelenmiş ve bu iki faktörün tümör içi immün sistemi nasıl etkilediğini bulmak hedeflenmiştir.

Yöntemler: Seröz over tümörü olan 37 hastada MMR proteinleri ve PD-L1 düzeylerine bakmak için immünohistokimyasal yöntemler kullandık. Bunlardan 14'ünde yüksek dereceli seröz karsinom, 6'sında düşük dereceli seröz karsinom, 8'inde borderline seröz tümör ve 9'unda seröz kistadenom vardı.

Bulgular: Çalışmamızda sadece dört olguda (%10,8) dMMR'yi gösteren MMR protein ekspresyon kaybı saptandı. MSI durumu ile tümör boyutu, over lokalizasyonu, Uluslararası Jinekoloji ve Obstetrik Federasyonu evresi veya PD-L1 ekspresyonu arasında anlamlı bir ilişki bulunamadı.

Sonuç: Bu çalışmada, MMR protein ekspresyonu kaybı prognostik parametreler veya PD-L1 ekspresyonu ile ilişkili saptanmadı. Bazı çalışmalarda tümörlerin MSI durumu çeşitli prognostik parametrelerle karşılaştırılmış olsa da aralarındaki ilişki için henüz bir fikir birliğine varılamamıştır. MSI ve PD-L1 ekspresyonu arasındaki etkileşimi anlamak, over kanserinde hedefe yönelik kişiselleştirilmiş tedavi yaklaşımlarına rehberlik edebilir.

Anahtar Kelimeler: Mikrosatellit instabilite, PD-L1, seröz over kanseri, immünohistokimya

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Introduction

Ovarian cancer is the second most prevalent form of cancer among gynecological malignancies and is the most lethal gynecological malignancy that affects women (1). The subtype of serous ovarian cancer is the most common within this category (2). Although early-stage diagnosis plays a crucial role in ovarian malignancies, patients frequently acquire diagnoses at advanced stages. (3). The prognosis for advanced ovarian cancer is significantly unfavorable, with diminished efficacy of standard treatments such as chemotherapy and radiotherapy. Therefore, advancing novel therapeutic methods, such as immunotherapy, has been paramount. Recent research has discovered that microsatellite instability (MSI) and programmed death cell ligand 1 (PD-L1) expression play a crucial role in how tumors interact with the immune system. MSI, which stands for defective DNA mismatch repair (dMMR), has attracted attention because it correlates with increased immunogenicity and improves responsiveness to immune checkpoint inhibitors in different types of cancer.

The PD-L1, a protein found on the surface of cancer cells, interacts with the PD-1 receptor on immune cells. This interaction suppresses the immune response and allows the cancer cells to avoid being attacked by the immune system. Understanding the complex connection between MMR protein expression and PD-L1 status in ovarian cancer is crucial for deciphering the immune system's characteristics and investigating potential treatment options. Nevertheless, the specific location and significance of dMMR and MSI status in ovarian cancer are not fully comprehended. The clinicopathologic characteristics of dMMR ovarian tumors are still not well understood, as there is conflicting information regarding the agreement between MMR deficiency and MSI status. Moreover, there is still ambiguity regarding the potential effectiveness of PD-1/PD-L1 inhibition in treating ovarian malignancies with MMR deficiency, as previous studies have only demonstrated mild responses in ovarian carcinomas (4).

The documented prevalence of MSI in ovarian cancer varies from 2% to 20% (2). Endometrioid and clear-cell ovarian carcinomas are the predominant subtypes of ovarian malignancies that have been found to display MSI, as reported in many publications (5). However, there is limited research explicitly addressing serous ovarian carcinomas with MSI.

This study's objective is to examine the frequency of dMMR and PD-L1 expression in ovarian serous tumors and assess their relationship with other prognostic factors.

Methods

Collection of Materials

Our pathology department diagnosed 37 instances between 2012 and 2020, including 14 high-grade serous carcinomas, six low-grade serous carcinomas, eight borderline serous tumors, and nine serous cystadenomas. These cases were selected based on their sufficient clinical knowledge. The hematoxylin and eosin (H&E)-stained case preparations were reassessed, and suitable blocks that accurately represented the tumor were chosen for immunohistochemical (IHC) investigation.

Compliance with Ethical Standards

All the authors declare that this study complied with the Declaration of Helsinki, as approved by the Karadeniz Technical University Ethics Committee (protocol number: 2020/251, date: 06.11.2020). Written informed consent was obtained for the study.

Immunohistochemistry (IHC) and Scoring

IHC experiments were conducted using the Ventana BenchMark Ultra, a fully automated staining system manufactured by Ventana Medical Systems, Inc. in the United States. Four micrometer thick slices were obtained from tissue blocks using a polymer-coated lamella to do this. After removing the paraffin, the IHC staining process was started.

The assessment of MMR protein expressions was conducted in the following manner: Positive staining or intact nuclear expression for each antibody was determined based on the existence of nuclear staining. The absence of nuclear staining in tumor cells was characterized as a lack of MMR protein expression. MMR protein expression is typically detected in lymphocytes and/or stromal cells, which serves as a positive internal control.

The outcomes are commonly categorized in the following manner:

1-Proficient Mismatch Repair (pMMR): This term describes tumors that show complete expression of all four MMR proteins. pMMR status signifies the operational state of the MMR system and the tumor's ability to correct DNA replication mistakes effectively.

2-Deficient Mismatch Repair (dMMR): This term describes tumors that lack the expression of one or more MMR proteins. An instance of dMMR classification occurs when a tumor exhibits the absence of MutL homolog 1 (MLH1) and postmeiotic segregation increased 2 (PMS2) expression while maintaining the expression of MutS homolog 2 (MSH2) and MutS homolog 6 (MSH6).

The PD-L1 antibody clone 28-8 was utilized. The PD-L1 expression was evaluated by scoring the sections based on the percentages of tumor cells showing entire circumferential or partial linear plasma membrane staining. A tumor is classified as PD-L1 positive when 5% or more of its cells show staining for PD-L1. The tonsillar tissue was utilized as the external positive control.

Statistical Analysis

The data analysis was conducted using the SPSS 22.00 statistical analysis tool. When comparing numerical variables between two independent groups, the Student's t-test is used if the normal distribution requirement is satisfied. If the normal distribution condition is not met, the Mann-Whitney U test is used instead. The ANOVA test was conducted by comparing at least three

groups to see if the normal distribution assumption was fulfilled. In contrast, the Kruskal-Wallis test was utilized if this assumption was not maintained. The chi-square test was applied to compare categorical data. A p-value of less than 0.05 was considered statistically significant.

Results

Clinicopathological Parameters

Fourteen, 6, 8, and 9 cases of high-grade serous carcinoma, low-grade serous carcinoma, borderline serous tumor, and serous cystadenoma were diagnosed, respectively. The International Federation of Gynecology and Obstetrics (FIGO) stage I/II was identified in 16 cases (high-grade 4 cases, low-grade 5 cases, borderline 7 cases). FIGO stage III/IV was determined in 12 cases (high-grade 10 cases, low-grade 1 cases, and borderline 1 cases). The characteristics of patients are summarized in Table 1.

Expression of PD-L1

Four cases (10.8%) (high-grade serous carcinoma) were identified as PD-L1 positive by IHC, as shown in Figure 1. There was no significant relationship between PD-L1 expression and tumor size (p=0.282), FIGO stage (p=0.053), or localization (p=0.276).

Expression of MMR proteins

The IHC identified four cases (10.8%) (3 in high-grade serous carcinoma and 1 in low-grade serous carcinoma) as dMMR.

Table 1. Characteristics of the study population

Characteristics	Number of patients	Percentage of patients (%)
Age at diagnosis		
<50	13	35.1
≥50	24	64.9
Histology		
High grade serous	14	38
Low grade serous	6	16
Borderline	8	22
Serous cystadenoma	9	24
FIGO state		
I, II	16	43
III, IV	12	32
Localization		
Right ovary	12	32
Left ovary	11	30
Bilateral	14	38
Tumor size		
<10cm	25	68
≥10cm	12	32
Total	37	100

FIGO: International Federation of Gynecology and Obstetrics

The expression loss distribution in immune markers in these cases was as follows (Table 2).

-Isolated loss in PMS2 in 3 cases is shown in Figure 2 (all the cases are in high grade).

-Loss in MLH1 and PMS2 expressions in 1 case (low grade)

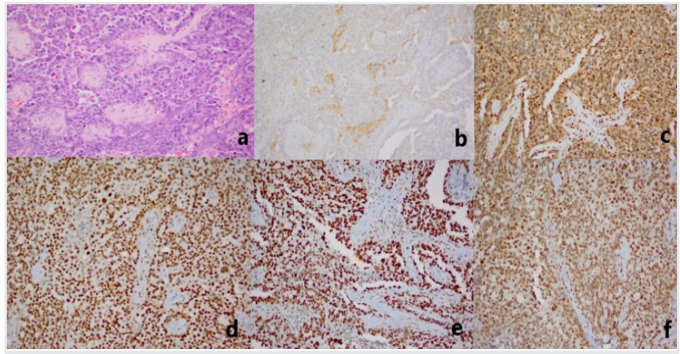

Figure 1. High grade serous ovarian carcinoma a) H&E x200, b) PD-L1x200 (positive staining), c) PMS2x200, d) MSH-2x200, e) MSH-6x200, f) MLH-1x200. No loss of MMR protein expression

H&E: Hematoxylin and eosin, PD-L1: Programmed death cell ligand 1, PMS2: Postmeiotic segregation increased 2, MSH: MutS homolog, MLH: MutL homolog, MMR: Mismatch repair

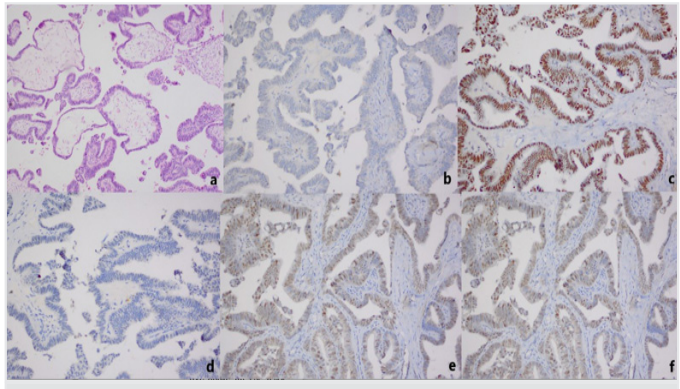

Figure 2. High grade serous ovarian carcinoma a) H&E x200, b) PD-L1x200 (negative staining), c) MSH-6x200, d) PMS-2x200 (loss of PMS-2 expression), e) MLH-1x200, f) MSH-2x200

H&E: Hematoxylin and eosin, PD-L1: Programmed death cell ligand 1, MSH: MutS homolog, PMS2: Postmeiotic segregation increased 2, MLH: MutL homolog

Table 2. The MMR expression status of cases with dMMR

	MLH1 loss	MSH2 loss	MSH6 loss	PMS2 loss
Case 1	-	-	-	+
Case 2	-	-	-	+
Case 3	-	-	-	+
Case 4	+	-	-	+

dMMR: DNA mismatch repair, MLH1: MutL homolog 1, MSH2: MutS homolog 2, MSH6: MutS homolog 6, PMS2: Postmeiotic segregation increased 2

-There was no significant relationship between dMMR and tumor size (p=0.582), localization (p=0.625), FIGO stage (p=0.464).

Relationship between dMMR and Expression of PD-L1

There was no significiant relationship between MSI status and expression of PD-L1 (p=1.000). While PD-L1 was positive in 4 of 37 cases which did not show loss of MMR protein expression.

Discussion

Our study, which examined how dMMR and PD-L1 expression were related in ovarian serous tumors, revealed a fascinating finding: there was no significant link between these two molecular markers. This finding prompts a comprehensive discussion of potential explanations and the broader implications for understanding the tumor microenvironment in ovarian cancer.

Ovarian serous tumors are known for their molecular heterogeneity, which can significantly impact the expression patterns of various biomarkers. The fact that our study found no link between dMMR and PD-L1 expression might show how different the molecular landscape was in this group of ovarian cancers. This variety can be caused by changes in underlying genes, tumor growth, and effects on the microenvironment. This is one reason why there isn't a single link between dMMR and PD-L1.

Understanding the lack of correlation between dMMR and PD-L1 in ovarian serous tumors is crucial for guiding therapeutic strategies. The absence of a direct correlation suggests that a comprehensive molecular profiling approach may be necessary to identify patients who may benefit from immune checkpoint inhibitors. When dMMR and PD-L1 don't match, looking into other immunotherapeutic targets and combination therapies tailored to each tumor's molecular makeup may be more helpful.

Vierkoetter et al. (7) found that patients with dMMR were younger than those with pMMR (mean age: 47 and 58 years, respectively) (p=0.0014). However, in our study, the four patients with dMMR were 67, 64, 63, and 58, with a mean age of 63. Contrary to the previous research, our findings indicated that cases with dMMR were older than those with pMMR.

Norquist et al. (8) identified MMR gene mutations in eight out of 1,915 patients with ovarian cancer, with 88% of these patients showing defects in the PMS2 and MSH6 genes. The tumor type in four patients with PMS2 mutations was high-grade serous carcinoma. Our study used MLH1, MSH2, MSH6, and PMS2 markers for IHC analysis. Three patients with MMR loss only had PMS2 protein expression loss, while one had both PMS2 and MLH1 loss.

In the study by Ryan et al. (9), MSI was most often found in the endometrioid subtype, but it was also found in high-grade serous ovarian carcinomas, which was a statistically important finding. Although not statistically significant, three out of four cases with MMR loss in our study were in the high-grade serous ovarian carcinoma subtype. More extensive studies ought to support this finding.

Study Limitations

Considering the temporal and spatial dynamics of dMMR and PD-L1 expression is essential. Tumor evolution and the dynamic nature of the immune response may lead to temporal variations in these molecular markers. Additionally, spatial heterogeneity within the tumor microenvironment can contribute to divergent expression patterns, complicating efforts to establish a straightforward correlation. More in-depth studies that look at both space and time may give us a better understanding of how dMMR and PD-L1 are connected in ovarian serous tumors.

The lack of a link could also mean that immune evasion in ovarian serous tumors isn't just dependent on the PD-L1/PD-1 axis in patients with dMMR. Alternative immune checkpoint pathways, tumor-intrinsic factors, or additional immune evasion mechanisms could have a role. Looking into other immunomodulatory molecules and pathways in the tumor microenvironment might reveal more layers of complexity in the immune response and help explain why dMMR and PD-L1 don't work together in this case (6).

Conclusion

This research contributes to our existing knowledge of the intricate molecular characteristics of ovarian serous tumors and highlights the significance of gaining a deeper understanding of the correlation between dMMR and PD-L1 expression. The absence of this link demonstrates the complexity of the interplay between the tumor and the immune system in ovarian cancer, emphasizing the importance of continued research to uncover the various mechanisms through which the immune system can overcome this challenge.

Ethics

Ethics Committee Approval: All the authors declare that this study complied with the Declaration of Helsinki, as approved by the Karadeniz Technical University Ethics Committee (protocol number: 2020/251, date: 06.11.2020).

Informed Consent: Written informed consent was obtained for the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.T., Concept: G.T., Design: G.T., Data Collection or Processing: G.T., M.E.E., Analysis or Interpretation: G.T., Literature Search: G.T., M.E.E., Writing: G.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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