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Clear cell carcinoma of the uterine cervix; an unusual HPV-independent tumor: Clinicopathological features, PD-L1 expression, and mismatch repair protein deficiency status of 16 cases

Uterin serviksin berrak hücreli karsinomu; HPV-ilişkisiz nadir bir tümör: 16 olgunun klinikopatolojik özellikleri, PD-L1 ekspresyon ve MMR protein ekspresyon kaybı durumları

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Abstract

Objective: Endocervical clear cell carcinoma (c-CCC) is a rare and HPV-independent adenocarcinoma type of cervix. Being usually resistant to conventional chemotherapy. Immunotherapy has recently been added as a preferred regimen as a second-line treatment option for programed cell death-ligand 1 (PD-L1)-positive or mismatch repair (MMR) deficient cervical carcinomas. In this study, clinicopathological features, PD-L1 expression, and MMR deficiency status of c-CCCs were investigated.

Materials and Methods: Sixteen c-CCC diagnosed cases were included in this study. PD-L1 expression was evaluated using two different PD-L1 clones (22C3 and SP263). MMR deficiency status of the cases was evaluated using four MMR proteins (MLH1, PMS2, MSH2, and MSH6).

Results: Most of the c-CCC cases were presented as FIGO Stage I (68.75%). PD-L1 expression in either tumoral or tumor-infiltrating immune cells (TILs) was present in 62.5% (10/16) and 69% (11/16) of the 22C3 and SP263 clones, respectively. Most of the cases with high TIL density were also positive for PD-L1. The PD-L1 expression rate was less than 50% in most of the cases and 12.5% of the cases shared extensive PD-L1 staining. Overall, MMR deficiency was observed in 31.25% of the cases. Most of the MMR-deficient cases (80%) were PD-L1 positive.

Conclusion: Although our study cohort is limited, we have shown that PD-L1 expression and MMR deficiency can be found in c-CCCs in variable degrees. These findings suggest that accompanying TIL density and MMR deficiency could be used as candidates for predicting PD-L1 positivity for c-CCCs. However, to indicate the clinical importance of these findings, objective treatment outcomes of cases treated with immunotherapy should be seen.

Keywords: Endocervical clear cell carcinoma, PD-L1 22C3, mismatch repair deficiency

Öz

Amaç: Endoservikal berrak hücreli karsinomlar (s-CCC), genellikle geleneksel kemoterapiye dirençli olan serviksin nadir bir HPV ilişkisiz adenokarsinom tipidir. İmmünoterapi yakın bir zamanda programlanmış hücre ölümü ligandı 1 (PD-L1)-pozitif veya mismatch-onarım (MMR) protein ekspresyon kaybı olan servikal karsinomların ikinci basamak tedavisinde tercih edilen bir rejim olarak eklenmiştir. Bu çalışmada, s-CCC'lerin klinikopatolojik özellikleri, PD-L1 ekspresyonu ve MMR eksikliği durumu araştırıldı.

Gereç ve Yöntemler: S-CCC tanısı koyulmuş olan 16 hasta bu çalışmaya dahil edildi. PD-L1 ekspresyonu iki farklı PD-L1 klonu (22C3 ve SP263) kullanılarak değerlendirildi. MMR eksikliği durumu dört MMR proteini ile (MLH1, PMS2, MSH2, MSH6) değerlendirildi.

PRECIS: The presence of PD-L1 expression and Microsatellite Instability in endocervical clear cell carcinomas predicts the immunotherapy may yield promising results in the treatment of endocervical clear cell carcinomas as well.

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Bulgular: S-CCC olgularının çoğu FIGO Evre I (%68,75) olarak prezente oldu. PD-L1 ekspresyonu, tümörde ya da tümörü infiltre eden lenfositlerde (TIL) 22C3 ve SP263 klonlarıyla sırayla olguların %62,5'inde (10/16) ve %69'unda (11/16) mevcuttu. Yüksek TIL yoğunluğuna sahip olguların çoğu PD-L1 ile de pozitifti. PD-L1 ekspresyon oranı çoğu olguda %50'den azdı ve olguların %12,5'i yaygın PD-L1 boyanması gösteriyordu. Genel olarak, olguların %32,25'inde MMR proteinlerinde ekspresyon kaybı gözlendi. MMR proteinlerinde ekspresyon kaybı olan olguların çoğu (%80) PD-L1 pozitifti.

Sonuç: Çalışma grubumuz sınırlı olmasına rağmen, PD-L1 ekspresyonu ve MMR proteinlerinde ekspresyon kaybının s-CCC'lerde değişen oranlarda bulunabileceğini gösterdik. Bulgular, eşlik eden TIL yoğunluğunun ve MMR protein ekspresyon kaybının, PD-L1 pozitifliğini tahmin etmek için bir aday olarak kullanılabileceğini düşündürmektedir. Ancak bu bulguların klinik öneminin gösterilebilmesi için immünoterapi ile tedavi edilmiş olguların objektif tedavi sonuçlarının görülmesi gerekmektedir.

Anahtar Kelimeler: Endoservikal berrak hücreli karsinom, PD-L1, 22C3, uyumsuzluk onarımı eksikliği

Introduction

Clear cell carcinoma (CCC) of Müllerian origin is a rare tumor with distinct histology that may occur in the ovary, vagina, uterus and cervix. Among carcinomas of the cervix, the most frequent histologic type is squamous cell carcinoma (SCC), which represents 75% of all cases. This is followed by endocervical adenocarcinoma (ECA), which accounts for 20-25% of all cases. ECA in fact represents a heterogeneous group of tumors with various etiologies, molecular drivers, morphologies, responses to treatment, and prognoses. ECA classification has recently been reorganized by the International Endocervical Adenocarcinoma Criteria and Classification and updated by the 2020 World Health Organization with separation into human papillomavirus (HPV)-associated (HPVA) and HPV-independent (HPVI) categories[1]. Cervical clear cell carcinoma (c-CCC) is one of the HPVI ECA types and accounts for only 3.3% of all ECAs[2]. Although the clinicopathological features of other Müllerian system-derived CCCs have been broadly studied, little is known about the clinicopathological features and optimal treatment strategies of c-CCCs due to their rarity.

C-CCCs is morphologically identical to their endometrial and ovarian counterparts, with solid, tubulocystic, and papillary architectures. Tumor cells are typically characterized by clear cytoplasm and hobnail nuclei, prominent cell membranes, hyperchromatic nuclei, and low mitotic rate. Oxyphilic, flat, and signet ring cells can be seen albeit rarely. Clear cells are round or polyhedral and contain abundant glycogen and occasionally hyaline globules[3,4].

The prognosis of c-CCC varies with stage, but the actual risk associated with this histology is unknown. Although most studies on ECA survival rates have not evaluated c-CCC separately, they have shown that HPVI ECAs have a worse prognosis than HPVAs[5].

The treatment approach c-CCC is consistent with the other cervical cancer types. Radical hysterectomy or trachelectomy, pelvic lymphadenectomy, +/- external beam radiotherapy (EBRT), and +/- brachytherapy constitute a standard treatment approach for early-stage cervical carcinoma (FIGO stages IA and IB1), while EBRT and systemic chemotherapy are the standard surgical treatment regimen for further stages (FIGO stages IB2, II, and III). According to the NCNN (National Comprehensive Cancer Network) version 1.2023 cervical cancer guidelines, pembrolizumab has been added as a preferred regimen as a second-line option for treating programed cell death-ligand 1 (PD-L1) positive or microsatellite instability-high (MSI-H)/mismatch-repair deficient (dMMR) tumors[6-8]. However, PD-L1 expression status in c-CCCs has been demonstrated in only a few studies in the English literature, and a successful immunotherapy response has recently been published as a case report[9-11].

As is known, four commercial PD-L1 expression assays linked to different PD-1/PD-L1 checkpoint inhibitors are currently available for the treatment of several cancer types 22C3, 28-8, SP142, and SP263. Among these, the 22C3 assay has received FDA approval as a "companion diagnostics" for the treatment of pembrolizumab in many cancer types, including cervical cancer[12].

Furthermore, MSI-H/dMMR is identified as a biomarker for immunotherapy efficacy and pointed to the potential use of immune checkpoint inhibitors in several cancer types including cervical cancer by the Keynote-158 trial[13]. PD-L1 expression, MSI status, and their correlation with clinicalopathologic features have already been investigated in other HPVA and HPVI-type cervical carcinomas in larger series[14]. However, the data regarding the situation for these biomarkers in c-CCCs are limited. In this study, we investigated the prevalence of PD-L1 expression (by using PD-L1 22C3 and SP263 assays) and MMR (by using MLH1, MSH2, MSH6, and PMS2 proteins) deficiency status and their relationship with the clinicalopathological features in our c-CCC series.

Materials and Methods

Case Selection and Clinicopathological Evaluation

A total of 16 primary c-CCC cases were included in this study. Among them, 14 cases were selected from the 105 ECA cases that were gathered previously for the design of the reproducibility of the new ECA classification study by our team[5]. After adding 2 new cases to expand the cohort, all available hematoxylin and eosin-stained (H&E) slides were reviewed by two pathologists to confirm the diagnosis and determine the optimal tumor-containing tissue block.

Diagnostic confirmation was made by identification of the classic morphologic features of c-CCC, including high-grade tumor cells with hobnail nuclei and prominent nucleoli in solid, papillary, and/or tubulocystic architectures. Napsin-A, p53, ER, PR immunohistochemical stains, and HPV-DNA in

situ hybridization (ISH) techniques were also applied for the confirmation of the morphologic diagnosis The presence of stromal tumor-infiltrating lymphocytes (TILs) was assessed independently on each slide. Stromal TILs are reported as a percentage of tumor stroma occupied by lymphocytes. TILs are classified into three groups using the following cut-off values: 10% (mild), 10-40% (moderate), and >40% (high).

Clinicopathological parameters, including age at diagnosis, presence of lymphovascular space invasion, nodal status, and International Federation of Gynecology and Obstetrics (FIGO) stage, were recorded from electronic medical records. It was also confirmed that the tumors originated from the endocervix by confirming that there was no tumor infiltration either in the ovary or in the endometrium.

Immunohistochemical Assessment

The 4-um-thick whole tissue sections were taken from formalin-fixed paraffin-embedded blocks. PD-L1 immunohistochemistry was conducted with two PD-L1 antibody clones on two different staining platforms. The SP263 antibody clone (Roche Diagnostics, Mannheim, Germany) was used on the Ventana Benchmark Ultra platform, and the 22C3 antibody clone (Agilent Technologies, Waldbronn, Germany) was run on a DAKO Autostainer Link 48 at the Koç University Hospital, Pathology Department (Istanbul, Turkey). All assays are referred to hereafter by the antibody clone used.

The combined positive score (CPS) and tumor proportion score (TPS) were used for evaluating PD-L1 positivity. CPS was calculated by dividing the total number of viable tumor cells by the number of cells stained with PD-L1 (including tumor cells, lymphocytes, and macrophages) and multiplying by 100. Only intra- and peritumoral immune cells were counted for scoring immune cells in the CPS system. Stromal immune cells from outside the tumor were not included. The percentage of viable tumor cells with partial or complete membrane staining at any intensity was used to calculate TPS. Cut-off score 1 was considered positive for CPS, and over 50 was considered as extensive staining[9,15]. For each stain, including PD-L1, appropriate positive and negative controls were included.

MSI status was evaluated by immunohistochemistry using DNA mismatch repair (MMR) proteins; MLH1 (1:200, Abcam, Cambridge, England), MSH2 (1:50, Roche, Mannheim, Germany), MSH6 (1:100, Roche, Mannheim, Germany), and PMS2 (1:100, Abcam, Cambridge, England). Simultaneous expression of four MMR proteins (MLH1, MSH2, MSH6, and PMS2) was considered "proficient DNA mismatch repair (pMMR)". Otherwise, "deficient DNA mismatch repair (dMMR)" is defined as the absence of at least one of the four indexes stated above. The normal expression was defined as nuclear staining within tumor cells, with expression in tumor-infiltrating lymphocytes as the positive internal control. The absence of nuclear staining within tumor cells despite concurrent positive labeling in internal nonneoplastic tissues was described as a loss of expression.

Napsin-A (mouse monoclonal, MSVA-112), estrogen receptor (ER, 1:50, monoclonal rabbit ab, clone SP1), progesterone receptor (PR, 1:50, monoclonal rabbit ab, clone 1E2), p53 (1:200, monoclonal mouse ab, clone DO7), and p16 (prediluted, monoclonal mouse ab, clone E6H4) were stained on an automatic immunostainer [Ventana Benchmark XT (Ventana Medical Systems, Tucson, AZ, USA)]. ER and PR stains were scored on a continuous quantitative scale based on the percentage of nuclear staining in tumor cells (0-100%). Focal or diffuse granular cytoplasmic staining was recorded as positive for Napsin-A. Diffuse, block-like staining of moderate or strong intensity was accepted as positive for p16, while patchy or no staining was interpreted as negative. p53 was noted as mutated either in the complete absence (null pattern) of staining or in the strong staining of >75% of tumor cell nuclei. The other positive staining rates were accepted as wild-type for p53[16]. The non-HPV associated status of the cases was already shown in a previous study using the HPV-DNA ISH technique (Detailed knowledge about the HPV-DNA ISH technique can be found in the referenced study)[5].

Immunohistochemical stains were initially independently assessed by two pathologists (P.B., O.C.E.). In the case of ambiguity, a consensus diagnosis was reached with a gynecopathologist with 20 years of experience in multi-head microscope (N.K.).

Statistical Analysis

To correlate PD-L1 expression with MMR status, clinicopathological features, and different TIL group, evaluations were made via 2-tailed χ^2 tests. For every analysis, statistical significance was set with p-value <0.05. IBM SPSS Statistics for Windows version 28.0 (IBM Corp., Armonk, NY, USA) was used for the analysis.

Results

Clinicopathological characteristics

The clinical characteristics of the cases are summarized in Table 1. The median age at diagnosis of 16 cases was 54 years (range 31-79 years), and 18% of the cases were under 40. There was no history of in utero diethylstilbestrol (DES) exposure in any of the patients. Most cases presented with vaginal bleeding and/or watery discharge. All patients underwent radical hysterectomy with bilateral salpingo-oophorectomy and pelvic-para-aortic lymph node dissection. The mean tumor size was 3.8 cm (1.0-8.0 cm). Three of the tumors invaded the upper two-thirds of the vagina (18.75%, 3/16) and one invaded the left parametrium (6.25%, 1/16). Two penetrates the vaginal surgical margin, one extended to the posterior cervical margin, and one concurrently had a tumor implant in the serosa of the sigmoid colon. The remaining tumors are confined to the cervix with a negative surgical margin (75%, 12/16). The initial FIGO stages for most patients were stages I and III (87.5%), except for one patient with stage IIA1 and one with stage IVA. The initial

Table 1. Clinicopathological characteristics of the cases

Parameters	n (%)
Age	
Average (range)	54 (31-79)
Tumor size (cm)	
Average (range)	3.8 (1-8)
FIGO Stage at diagnosis	
Stage I	11 (68.75)
IA1	2 (12.5)
IA2	0 (0)
IB1	1 (6.25)
IB2	3 (18.75)
IB3	5 (31.25)
Stage II	1 (6.25)
IIA1	1 (6.25)
IIA2	0 (0)
IIB	0 (0)
Stage III	3 (18.75)
IIIA	0 (0)
IIIB	0 (0)
IIIC1	1 (6.25)
IIIC2	2 (12.5)
Stage IV	1 (6.25)
IVA	1 (6.25)
IVB	0 (0)
Silva Pattern invasion	
A	0 (0)
B	1 (6.25)
C	15 (93.75)
TILs	
Mild	6 (37.5)
Moderate	3 (18.75)
High	7 (43.75)
LVI	
Positive	8 (50)
Negative	8 (50)
PNI	
Positive	2 (12.5)
Negative	14 (87.5)
LNM	
Negative	12 (75)
Positive (pelvic)	2 (12.5)
Positive (paraaortic)	2 (12.5)

TILs: Tumor-infiltrating lymphocytes, LVI: Lymph vascular invasion, LNM: Lymph node metastasis, PNI: Perineural invasion

FIGO stages were distributed as follows: stage IA1: 12.5% (2/16), IB1: 6.25% (1/16), IB2: 18.75% (3/16), IB3: 31.25% (5/16), IIA1: 6.25% (1/16), IIIC1: 6.25% (1/16), IIIC2: 12.5% (2/16), and IVA: 6.25% (1/16). Lymphovascular invasion (LVI) was in eight (50%) cases and absent in eight (50%) cases. LVI was not seen in the early-stage tumors (FIGO Stages IA and IB1). Lymph node metastasis (LNM) at the time of diagnosis was in 4 (25%) and absent in 12 (75%). Two of these were pelvics (50%) and two were paraaortic (50%) lymph nodes. Survival data of 14 cases were available. The mean follow-up time of the cases was 36 months. Two of the cases died on the 13th and 18th months after surgery (Cases are numbered 1 and 10, respectively, according to their position in Table 2). Other cases were still alive with no recurrence or metastasis in the following period.

Histologically, 6 (37.5%) tumors had an exophytic polypoid appearance with mostly superficial infiltration of the endocervical mucosa. One case was restricted to the endocervical epithelium, with a polypoid appearance (5 cm in size) and no evident cervical stormal invasion. Fifteen cases showed Silva pattern-C infiltration (93.75%), and one case had a Silva pattern-B infiltration as there was no significant cervical stormal invasion (6.25%)[17].

The histological appearance of the cases was similar to other gynecological system origins, with an ordinary CCC appearance (Figure 1A). The tumor cells were arranged in a tubullocystic, papillary, or solid architecture (often a mix to varying degrees) and were surrounded by cells with clear (intracytoplasmic glycogen), eosinophilic, granular, and sometimes hobnailed cytoplasm with minimal stratification. In some cases, pseudonuclear inclusions and hyaline globules were seen. One case consisted of well-differentiated cysts lined by a single flattened epithelial layer on a hyalinized stroma that could easily be mistaken for benign entities as defined. TILs were seen

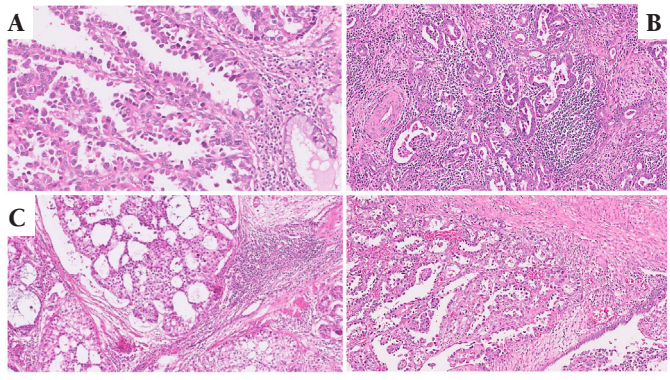

Figure 1. A-D) Histological appearance of clear cell carcinomas (CCC) and accompanying varying amounts of tumor-infiltrating lymphocytes (TILs). **A-** The histologic appearance of classic CCC from the high-power view (H&E x200), **B-** High TIL infiltration (H&E, x100), **C-** Moderate TIL infiltration (H&E, x100), **D-** Mild TIL infiltration. A few TIL clusters can be seen in the right part of the figure (H&E, x100)

in all cases at different rates (Figure 1B-D). However, stromal high TIL was observed in 7 (43.75%) cases. Moderate and mild TIL was observed in 18.75% and 37.5% of cases, respectively. Carcinoma *in situ* foci defined for CCCs in the literature were not observed in any of the cases[18]. Immunohistochemically, all tumors were Napsin-A positive and wild-type with p53. Conveniently, ER, PR, or P16 expression was not seen in any case.

Mismatch Repair Protein Deficiency

Thirty-one percent (5/16) of the c-CCC cases demonstrated MMR deficiency (Table 2). Two of them showed a dual loss of MSH2 and MSH6 (Figure 2), 1 showed a dual loss of MLH1 and PMS2, 1 showed a triple loss of MLH1, PMS2, and MSH6, and 1 showed a single loss of MSH6 proteins. Forty percent (2/5) of dMMR c-CCC cases showed extensive PD-L1 positivity in both TPS and CPS scores. PD-L1 positivity was seen in 80% of dMMR cases. Eighty percent (4/5) of dMMR cases were accompanied by a high rate of TILs. The remaining dMMR case exhibited no PD-L1 expression in tumor cells or TILs. The relationship between PD-L1 expression and MMR status was not statistically significant for either tumor (p=0.3) or combined tumor and inflammatory cells (p=1) (Table 3).

Tumoral and Peritumoral Immune PD-L1 Expression

PD-L1 expression status and their relationship with the MMR results for each case are shown in Table 2. In 69% (11/16) of cases with SP263 clones and 62.5% (10/16) of cases with 22C3 clones, either the tumor (TPS) or immune cells like lymphocytes and macrophages that were infiltrating the tumor (CPS) were stained with PD-L1. PD-L1 expression was seen in 56.25% (9/16) of the cases based on TPS in both clones. Both PD-L1 SP263 and 22C3 clones showed perfect accordance with TPS. For CPS also, concordance was excellent, except for one case that was considered negative for the SP263 clone

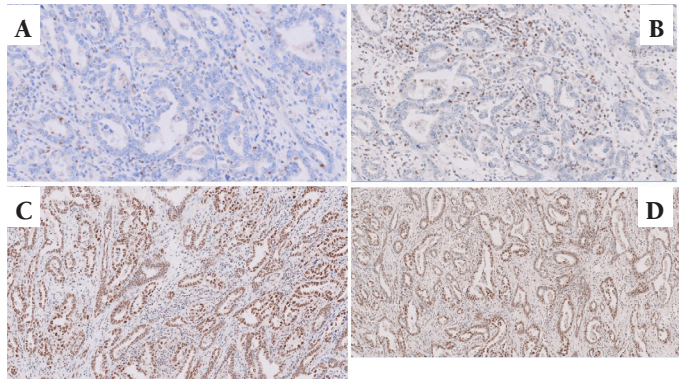

Figure 2. A-D) The microsatellite instability (MSI) status of case no 2. **A-** Concurrent loss of MSH-6 in tumor cells (IHC; x200), **B-** Concurrent loss of MSH-2 in tumor cells (IHC; x200), **C-** Intact MLH-1 expression (IHC; x100), **D-** Intact PMS-2 expression (IHC; x100)

Table 2. Detailed TIL status, PD-L1, and MMR IHC expression results of the cases

Case no	PD-L1/SP263		PD-L1/22C3		TILs	MMR IHC			
	TPS (%)	CPS (%)	TPS (%)	CPS (%)	Score	MLH1	PMS2	MSH2	MSH6
1	90	83	90	70	H	Loss	Loss	p	Loss
2	90	65	90	60	H	p	p	Loss	Loss
3	5	20	10	35	H	p	p	Loss	Loss
4	2	1	2	1	H	p	p	p	Loss
5	-	-	-	-	Mi	Loss	Loss	p	p
6	-	5	-	-	H	p	p	p	p
7	-	-	-	-	Mod	p	p	p	p
8	-	-	-	-	Mi	p	p	p	p
9	-	-	-	-	Mi	p	p	p	p
10	3	5	1	2	Mod	p	p	p	p
11	1	3	5	10	H	p	p	p	p
12	-	-	-	-	Mi	p	p	p	p
13	10	7	2	1	Mod	p	p	p	p
14	5	2	2	5	Mi	p	p	p	p
15	-	30	-	30	H	p	p	p	p
16	10	9	10	9	Mi	p	p	p	p

First 5 cases have a loss of expression (dMMR) with at least one of the MMR: Mismatch repair, IHC: Immunohistochemistry, TPS: Tumor proportion score, CPS: Combined positive score, TILs: Tumor-infiltrating lymphocytes, (-): Negative, p: proficient DNA mismatch repair (pMMR), Loss: deficient DNA mismatch repair (dMMR), H: High, Mod: Moderate, Mi: Mild

Table 3. Correlation between PD-L1 expression (according to the PD-L1 22C3 clone) and the clinicopathological characteristics of the cases

Variable	n (%)	PD-L1 expression TPS			n (%)	PD-L1 expression CPS		
		Positive	Negative	p-value		Positive	Negative	p-value
MMR IHC				0.3				0.58
dMMR	5 (31.3)	4 (25)	1 (6)		6 (37.5)	5 (31.25)	1 (6.25)	
pMMR	11 (68.7)	5 (31)	6 (38)		10 (62.5)	6 (37.5)	4 (25)	
TILs				0.35				0.061
Mild	6 (37.5)	2 (12.5)	4 (25)		6 (37.5)	2 (12.5)	4 (25)	
Moderate	3 (18.7)	2 (12.5)	1 (6.25)		3 (18.7)	2 (12.5)	1 (6.25)	
High	7 (43.8)	5 (31.25)	2 (12.5)		7 (43.8)	6 (37.5)	1 (6.25)	
Age (years)				0.61				0.11
<54	8 (50)	4 (25)	4 (25)		8 (50)	4 (25)	4 (25)	
⩾54	8 (50)	5 (18.8)	3 (31.3)		8 (50)	7 (43.8)	1 (6.3)	
Tumor size (cm)				0.13				0.59
<3.8	8 (50)	3 (18.8)	5 (31.3)		8 (50)	5 (31.3)	3 (18.8)	
⩾3.8	8 (50)	6 (37.5)	2 (12.5)		8 (50)	6 (37.5)	2 (12.5)	
FIGO stage				0.83				0.60
I-II	11 (68.8)	6 (37.5)	5 (31.3)		10 (62.5)	8 (50)	2 (12.5)	
III-IV	5 (31.3)	3 (18.8)	2 (12.5)		6 (37.5)	4 (25)	2 (12.5)	
LVI				0.61				0.60
Positive	8 (50)	5 (31.3)	3 (18.8)		8 (50)	6 (37.5)	2 (12.5)	
Negative	8 (50)	4 (25)	4 (25)		8 (50)	4 (25)	4 (25)	
LNM				0.77				0.60
Positive	4 (25)	2 (12.5)	2 (12.5)		4 (25)	2 (12.5)	2 (12.5)	
Negative	12 (75)	7 (43.8)	5 (31.3)		12 (75)	8 (50)	4 (25)	

MMR: Mismatch repair, IHC: Immunohistochemistry, pMMR: proficient DNA mismatch repair, dMMR: deficient DNA mismatch repair, TILs: Tumor-infiltrating lymphocytes, LVI: Lymph vascular Invasion, LNM: Lymph node metastasis

(which was 5% positive for the 22C3 clone) (Table 2). Two of the cases showed extensive PD-L1 staining in both clones (18%; 2/11) (Figure 3). Most of the remaining PD-L1-positive cases showed tumoral staining in less than 50% of cells (82%; 9/11). The mean PD-L1 expressing rates for CPS were 16% for SP263 and 15.4% for 22C3. PD-L1 positivity was seen only in TILs in 2 cases (18%; 2/11), with one where positivity was seen only on the SP263 clone (5% PD-L1 staining rate).

Relationship Between PD-L1 Expression and Clinicopathological Features

The relationship between PD-L1 expression and clinicopathological parameters of the patients is shown in Table 3.

There was no correlation between CPS/TPS with age (p=0.61/p=0.11), tumor size (p=0.13/p=0.59), lymphovascular invasion (p=0.60/p=0.61) or lymph node metastasis (p=0.60/p=0.77) based on PD-L1 expression. There was no correlation between PD-L1 expression and FIGO stage as well (p=0.60/p=0.83). PD-L1 expression was higher in both TPS and CPS with high amounts of TIL. However, statistical significance was not found (CPS, p=0.061, TPS, p=0.35).

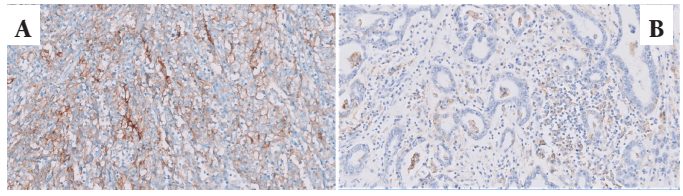

Figure 3. A- Extensive PD-L1 staining (with the 22C3 clone) in tumor and immune cells of case no 2 (TPS 90%, CPS 60%) (IHC; x200); **B-** A lesser degree of PD-L1 staining (with the SP263 clone) in tumor and immune cells of case no 3 (TPS 5%, CPS 20%) (IHC; x200)

Discussion

Immune checkpoints such as programmed cell death 1 (PD-1) and its ligand (PD-L1) are critical in antitumor immunity, and blocking them has been demonstrated to enhance

outcomes in patients with numerous types of malignancies[19]. Pembrolizumab, a PD-1 inhibitor, has been approved for the treatment of patients with PD-L1-positive cervical cancer that is locally progressed, recurrent, or metastatic[20]. In this study, a objective was to evaluate PD-L1 expression in c-CCC.

The CCC of the cervix accounts for approximately 3.3% of ECAs, which is further reduced if SCCs are also included in the cohort. Therefore, there is a paucity of data in the literature regarding the prognostic indicators, clinical outcomes, and treatment strategies of c-CCC. According to recent studies, the prognosis of early-stage c-CCC is similar to that of other types of cervical cancer. Advanced-stage and lymphatic involvement are associated with worse survival for both progression-free survival (PFS) and overall survival (OS) for c-CCC[21-25]. According to Liu et al.[24], the 5-year OS for FIGO stages IB to IIA and stage IIB to IIIC was 95.7% and 46.2%, respectively. Thomaset al.[25] have similarly found that the presence of positive lymph nodes has a negative impact on 5-year PFS (31% vs. 92%, p<0.001) and 5-year OS (80% vs. 100%, p=0.02) in stage I and IIA c-CCC patients. Stolnicu et al.[26] have recently compared the survival outcome between c-CCC and ECA and found a significant difference in 5- and 10-year OS between c-CCC and HPVA ECA, whereas no significant difference was shown between c-CCCs and gastric-type ECAs. Moreover, they emphasize the importance of the stage in OS with their results; They stated that OS in stage I CCC was 85.3% at both 5 and 10 years, while it was 39.7% at 5 years and 0% at 10 years in stages II to IV (p<0.001)[26].

Despite variations in survival and treatment responses between cervical HPVA and HPVI carcinomas, there is currently no therapeutic difference between SCCs and adenocarcinomas, including c-CCCs[27,28]. Radical surgery combined with targeted adjuvant therapy may cure early-stage disease. However, more advanced diseases are treated with EBRT and systemic chemotherapy[6]. According to the NCNN Version 1.2023 guidelines for cervical cancer treatment, concurrent chemoradiation is generally the primary treatment choice for stages of IB3 to IVA disease. In 2020, the FDA approved the addition of the PD-1 inhibitor pembrolizumab to the treatment of PD-L1-positive (CPS1)- or dMMR patients whose disease progresses after chemotherap[29-31]. Keynote-158 (pembrolizumab)[13], Empower-Cervical-1 (cemiplimab)[32], and Keynote-826 (pembrolizumab)[7] were pivotal studies that indicated immunotherapy enhanced overall survival in both post-platinum failure and frontline persistent, recurrent, or metastatic PD-L1 positive cervical cancer patients.

According to the Keynote-158 trial, the overall response rate was 14.4% in PD-L1-positive patients. Median PFS and OS for PD-L1-positive patients were 2.1 and 11 months, respectively. However, no responses were observed in patients with PD-L1-negative tumors. Regarding safety, 4.1% of patients stopped treatment because of treatment-related adverse events (including hepatitis, severe skin reactions, and adrenal insufficiency)[13].

The Empower-Cervical-1 study investigated the therapeutic efficacy of cemiplimab (PD-1 inhibitor) in 608 patients (304 of the patients randomly received cemiplimab and 304 received chemotherapy) with recurrent or metastatic cervical cancer. Although only a small portion of their patients could have their PD-L1 expression assessed, cemiplimab had a longer median overall survival than the chemotherapy group. (13.9 vs. 9.3 months) among the PD-L1 positive patients (≥1%). The median overall survival rates were 7.7 and 6.7 months with cemiplimab and chemotherapy in PD-L1-negative patients, respectively. According to the results, objective responses to cemiplimab were observed in 18% (≥1%) of PD-L1 positive patients and 11% (<1%) of PD-L1 negative patients. In the overall population, an objective response was obtained in 16.4% of patients in the complimab group compared with 6.3% in the chemotherapy group. This means that PD-L1-positive patients generally have an increased overall survival benefit. However, it can be concluded that PD-L1-negative patients also have an overall survival benefit with cemiplimab as or slightly better than patients receiving chemotherapy. Of their complimab and chemotherapy-received cohorts, 45% and 53.4% had grade 3 or higher adverse events, and 15.7% and 0.7% had immune-related adverse events, respectively[32].

The relative benefit of adding pembrolizumab (PD-1 inhibitor) to platinum-based chemotherapy with or without bevacizumab in PD-L1-positive metastatic or unresectable cervical cancer patients was investigated in 548 patients by Keynote-826 (pembrolizumab) study[7]. According to their results, progression-free (10.4 vs. 8.2 months) and overall survival (24-month estimate of patients alive, 53.0% vs. 41.7%) were significantly longer with pembrolizumab than with the placebo group. According to their study's adverse event data, 42.4% of patients in the placebo group and 49.8% of patients who received pembrolizumab experienced major adverse events. Only hypothyroidism (18.2% vs. 9.1%) and a lower white blood cell count (12.1% vs. 7.1%) posed a greater risk in the pembrolizumab group.

PD-L1 protein expression is currently used as a predictive biomarker for checkpoint therapy in cervical cancer. However, the heterogeneous expression tendency of the PD-L1 protein makes this method suboptimal. Therefore, the PD-L1 protein expression rate may not be directly associated with prognostic significance and treatment response. The patient selection according to PD-L1 protein expression excludes potential patients for whom checkpoint therapy could be effective. Rotman et al.[33] investigated the tumoral PD-L1 expression heterogeneity for cervical cancer. According to their results, 27% of cases had heterogeneity between different tumor cores based on the percentage of positive tumor cells. Additionally, for comparison, they also applied the RNAish technique and observed heterogeneity in 11% of the cases. Their results showed that core biopsies can consequently lead to false negative results

and the RNAish technique could serve as a better biomarker than IHC detection.

In this study, the PD-L1 expression status of c-CCCs was investigated using two different PD-L1 clones (SP263 and 22C3). PD-L1 expression scores were almost completely similar between the two clones. According to the results of a meta-analysis of the diagnostic accuracy of the PD-L1 IHC assays, the diagnostic sensitivity of the 22C3 was higher than the SP263 assay[34]. The fact that our results between the two clones were almost similar for both TPS and CPS scores may be due to the small number of patients.

PD-L1 expression in SCC and ECA has been previously reported[35,36]. According to their results, SCC had significantly higher PD-L1 expression positivity in tumor cells than ECAs (5% cut-off). Omenai et al.[35] recently published the PD-L1 expression profiles of 183 cervical cancer patients, irrespective of their histological type. According to their results, PD-L1 positivity was seen in 57.4% of the cases (58.7% in SCCs and 50% in ECAs). Song et al.[9] shared their results on PD-L1 expression and immune stormal features in HPVI cervical adenocarcinomas. According to their results, PD-L1 expression was seen in 58.3% (7 of 12) of c-CCCs. Moreover, they also found that PD-L1-positive cases (CPS ≥1) showed worse PFS and OS than PD-L1-negative cases. However, data in the literature regarding the PD-L1 expression status of CCCs, the prognostic effect of this expression, and their response to treatment are quite limited. Zong et al.[10] investigated the expression of different immune checkpoint proteins in c-CCCs. They found that 22% of cases had PD-L1-positive tumor cells (CPS ≥1).

Diffuse PD-L1 expression was observed in 18% of our cohort, and the mean PD-L1 expression rate in our c-CCC series was 16%.

Even not statistically significant high TIL density was seen more frequently in PD-L1-positive cases. PD-L1 positivity has been shown to be associated with the number of TILs in many tumor types, including cervical carcinoma[37]. As is known, TIL density is likely related to immunotherapy response[38]. Similarly, Song et al.[9] showed a significant association between high TIL percentage and CPS or TPS-based PD-L1 expression in their c-CCC cohorts. Additionally, PFS and OS were significantly poorer for PD-L1-positive subjects in their group than for PD-L1-negative cases. Unfortunately, we could not perform a survival analysis due to the short follow-up period in our series and the low number of cases.

Another aim of our study was to investigate the MMR deficiency status in c-CCCs, as the presence of MSI is one of the predictors of anti-PD1/PD-L1 immunotherapy response[39,40]. Anti-PD-L1 drugs can enhance survival, particularly in dMMR malignancies[41].

According to our results, 80% of the dMMR cases were also PD-L1 positive and 80% of the dMMR cases had high TILs. In a recent study involving 39 ECA cases, 2 of which were c-CCC, 15% of the cases were dMMR (all usual type), and 1 dMMR case was PD-L1 positive[42]. Song et al.[9] reported an MMR deficiency status of 16% in their c-CCC cohort (2/12 cases). Both were PD-L1-positive as well. Since the number of studies on the MSI status of c-CCCs is very limited, further studies with larger cohorts will be needed to validate these findings.

Study Limitations

This study has inherent limitations due to the limited sample size owing to the rarity of the disease. As a result, the generalizability of the current findings is limited.

Conclusion

However, our results are valuable in showing that c-CCCs can have PD-L1 expression and MMR deficiency. This shows that c-CCC cases, which are tumor types resistant to conventional chemotherapy, are candidates for immunotherapy similar to other cervical cancer types.

Ethics

Ethics Committee Approval: This study was approved by the ethics review board of Koç University (approval number: 2023.120.IRB2.028, date: 06.04.2023).

Informed Consent: Informed consent was obtained from all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Conceht: P.B., N.K., Design: P.B., N.K., Data Collection or Processing: P.B., Ö.C.E., Ö.Ö., A.N.H., N.K., Analysis or Interpretation: P.B., N.K., Literature Search: P.B., Ö.C.E., Writing: P.B.

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