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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Evaluation of PD-L1 expression in recurrent nonmetastatic sacral chordomas – a retrospective study

Tulay Zenginkinet[1], Erhan Okay[2], Ayse Nur Toksoz Yildirim[1], Ajkut Celik[2], Yalcin Turhan[3], Korhan Ozkan[2], Muhlik Akyurek[4]

[1]Istanbul Goztepe Prof. Dr. Suleyman Yalcin City Hospital, Department of Pathology, Istanbul, Turkey;
[2]Istanbul Goztepe Prof. Dr. Suleyman Yalcin City Hospital, Department of Orthopedics, Istanbul, Turkey;
[3]Duzce University, Faculty of Medicine, Department of Orthopedics and Traumatology, Duzce, Turkey;
[4]Maria-Josef-Hospital Greven, Clinic for Trauma Surgery and Orthopedics, Greven, Osnabrück, Germany

SUMMARY

Introduction/Objective Chordomas are rare tumors of a notochordal origin. Wide surgical resection is recommended for treatment. However, it is associated with a high risk of morbidity and mortality. Additionally, these tumors are resistant to chemotherapy. Thus, targeted therapy is needed for the treatment of chordomas. Programmed death ligand 1 (PD-L1) is a promising target for cancer treatment. Here, we investigated PD-L1 expression in patients with chordoma in a single-center study.

Methods Formalin-fixed paraffin-embedded blocks were evaluated for immunohistochemical analysis to evaluate PD-L1 expression. Clinicopathological variables, such as sex, age, and follow-up data (recurrence and outcome), were retrospectively collected from the patients' medical records.

Results Ten patients diagnosed with sacral chordoma in a single institution between December 2015 and November 2021 were included in this study. The median patient age was 57 years and the median follow-up period was 40 months. The surgical margins were negative in all cases, without any preoperative medical treatment. Four of the ten patients showed PD-L1 positivity on immune cells. These patients showed local recurrence, without metastasis. In these cases, the median time to local recurrence was 15 months. All the patients with the disease were alive.

Conclusion This study demonstrated that PD-L1 positivity in immune cells can be used as a predictive marker for local recurrence at the time of surgical treatment. This can potentially be used to determine the necessity to administer immunotherapy.

Keywords: chordoma; wide surgical resection; PD-L1; immunotherapy

INTRODUCTION

Chordomas are locally destructive, slowly enlarging tumors. The incidence of chordoma is estimated to be one per million per year in the United States and Europe [1]. These rare, primary tumors are derived from neural crest cells. They are expansile lytic lesions located in the midline of vertebral bodies.

These tumors are difficult to treat, with a very low rate of complete recovery. In advanced cases, chordomas invade the normal fascial barriers. Wide surgical resection is accepted as the primary line of treatment; however, it can result in high morbidity [2]. Neurological sacrifice causes bowel, bladder, and sexual dysfunction. Metastasis is rare, but recurrences have a poor prognosis, which makes the treatment more complicated.

Several multidisciplinary treatment strategies can be applied. This is particularly crucial when wide resection is not feasible. Therefore, the treatment strategies for chordomas should be formulated on a case-by-case basis. With advances in diagnostic and treatment options, promising new treatment strategies are being developed for chordoma. These include chemotherapy, imatinib treatment, adjuvant radiotherapy, and proton-beam therapy [3]. No standard effective adjuvant therapy regimens are currently available for chordomas treatment. In addition, the effect of these treatments on survival remains unclear. Such treatments, if effective, can avoid marginal excision, thus evading the morbidity risk.

Chordomas are resistant to radiation and chemotherapy [4]. New therapeutic options include the use of imatinib, which is an inhibitor of the platelet-driven growth factor receptor beta. It has been found to be effective in treating chordomas. In future, targeted therapy and immunotherapy for treating bone and soft tissue tumors have the potential to further improve prognosis.

Programmed cell death protein 1 (PD-1) is a candidate biomarker for chordomas [5]. PD-1 and programmed death ligand 1 (PD-L1) were discovered in 1992. It plays an important role in immune surveillance during cancer. Along with the activation of cytotoxic T lymphocytes, the balance of positive and negative signals is also important for the development

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Correspondence to:
Erhan OKAY
Istanbul Goztepe Prof. Dr.Suleyman Yalcin City Hospital
Department of Orthopedics and Traumatology
Egitim Mahallesi Fahrettin Kerim Gokay Caddesi
34722 Kadıkoy
Istanbul
Turkey
erhanokay@yahoo.com

of antitumor immunity. Similar to surface molecules, cytotoxic T-lymphocyte antigen-4 and PD-1 show negative signals. PD-1 is an inhibitory receptor, which belongs to the CD28 family and plays an important role in tumor immune escape. PD-1 and PD-L1 are the targets of new-generation drugs and can potentially be used for the treatment of many types of cancers. However, only two studies have evaluated the PDL1 receptor status in chordoma, thus requiring further research to prove its therapeutic efficacy. This study aimed to assess PD-L1 expression in patients with sacral chordomas.

METHODS

A retrospective review was conducted between 2016 and 2020 for all cases with histologically proven chordoma. Ten cases of sacral chordoma were identified. The diagnosis was made based on typical morphological features, S100/cytokeratin, and brachyury expression (Figure 1). Resected materials, including whole-tumor sections, were analyzed. The patients had not received treatment earlier. Adjuvant treatment was administered in patients with recurrence.

Clinicopathological characteristics, such as sex, age, and follow-up data (recurrence and outcome), were retrospectively collected from the patients' medical records.

Tissue samples obtained from resected specimens were used to prepare formalin-fixed paraffin-embedded blocks. Four-micron thick sections were obtained from these blocks for immunohistochemical analysis. General evaluation was performed using hematoxylin-eosin-stained slides. Necrosis, muscle invasion, surgical margins, and extracellular myxoid matrix were evaluated. Slides were stained with PD-L1 antibody (Cell Signaling / E1L3N). Immunohistochemical staining was performed as per the protocol provided by Cell Signaling Technology. PD-L1 expression in tumors and immune cells was also evaluated. Immunoreactivity for PD-L1 expression was evaluated in tumor cell membranes and immune cells by two blinded authors (ANY and TZ). PD-L1 results were separately evaluated in both tumor and immune cells in the microenvironment for each tumor component. PD-L1 expression was scored as positive when membranous staining was present in ≥ 5% of the population. Ethical committee approval was not necessary for discussion with the board. This study was conducted on human tissue samples preserved in the archives of the pathology department. Statistical analysis was not performed due to small sample size.

The study was conducted at the Istanbul Medeniyet University Medical School, Goztepe Training and Research Hospital according to the institutional ethical standards of the Helsinki Declaration.

RESULTS

Patient data are detailed in Tables 1 and 2. All cases had conventional chordomas. The median patient age was 57 years (range: 31–67 years). The median follow-up period was 40 months (range: 24–110 months). The surgical margins were negative in all cases and adjuvant therapy was not administered. Among the 10 cases considered in this study, PD-L1 on immune cells was positive in three patients. These patients experienced local recurrence with a median time of 15 months (range: 11–18 months). All the patients with the disease were alive. No preoperative chemotherapy or radiotherapy was administered to any of the patients. Chemotherapy and radiotherapy were administered in cases of metastasis and recurrence.

All cases showed muscle invasion. The resection margin was negative in all cases. Lymphocyte infiltrates were present in all the cases. The expression of PD-L1 by tumor cells was negative in all the cases (Figures 1 and 2). However, the expression of PD-L1 by immune cells varied in different cases. Positive expression in immune cells was observed in cases with local recurrence**.** Intratumoral lymphocytes were present in all specimens; however, only four positively stained for PD-L1 (Figure 3). Tumor margins were negative in all cases.

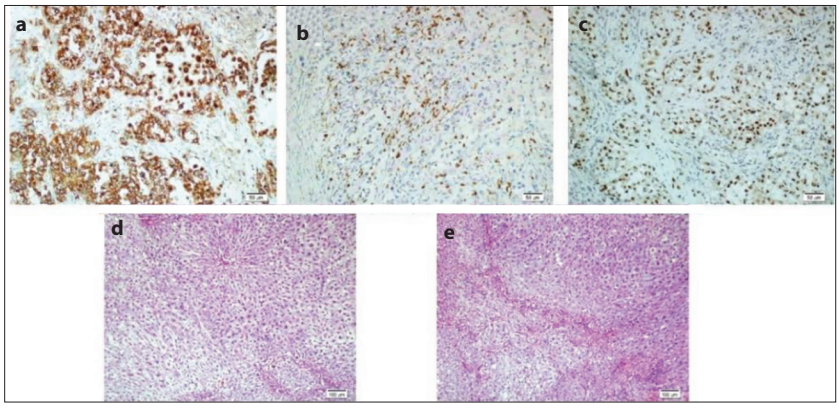

Figure 1. Immunohistochemical results of the tissue sample from the patient (Case 1); a – epithelial membrane antigen; b – S100 are expressed in conventional chordoma; however, they are usually negative in poorly differentiated chordoma; c – brachury is a nuclear stain, which is highly specific for chordoma; it is also positive in poorly differentiated chordoma; d–e – on hematoxylin and eosin-stained sections, tumor cells consist of dense epithelioid sheets in an extracellular myxoid matrix; cells are epithelioid with abundant clear (glycogen) to eosinophilic cytoplasm which may be have a bubbly/vacuolated appearance (physaliphorous cells)

DISCUSSION

Chordomas were first described by Virchow [6]. These slow-growing tumors present with pain and neurological dysfunction, secondary to obstruction of the lesion. Chordomas can occur at any location in the axial skeleton. Those involved in the sacral region can cause pain, urinary and/or bowel dysfunctions, and neuropathy. Chordomas are poor responders to chemotherapy; *en bloc* resection is the most effective method for local control.

Table 1. Clinicopathologic findings of all cases

Cases	Age (years), gender	Localization	Histologic subtype	Grade (high/low)	Metastasis	Recurrence	Time to recurrence (months)	Follow-up (months)	Tumor size (cm)
Case 1	63, Male	Sacrum	Conventional	Low	Negative	Negative		44	> 5 cm
Case 2	67, Male	Sacrum	Conventional	Low	Negative	Negative		24	> 5 cm
Case 3	31, Male	Sacrum	Conventional	High	Negative	Positive	11	36	> 5 cm
Case 4	52, Male	Sacrum	Conventional	High	Negative	Positive	15	63	> 5 cm
Case 5	62, Male	Sacrum	Conventional	Low	Negative	Negative		24	> 5 cm
Case 6	57, Male	Sacrum	Conventional	High	Negative	Negative		25	> 5 cm
Case 7	41, Male	Sacrum	Conventional	Low	Negative	Negative		73	> 5 cm
Case 8	57, Male	Sacrum	Conventional	High	Negative	Positive	18	110	> 5 cm
Case 9	65, Male	Sacrum	Conventional	High	Negative	Positive	16	82	> 5 cm
Case 10	47, Female	Sacrum	Conventional	Low	Negative	Negative		24	> 5 cm

Table 2. Pathologic findings of all cases

Cases	Age (years), gender	Tumor necrosis	Muscle invasion	Surgical margin	EMA	S100 protein	Brachyuria	PD-L1 expression		Status
								Tumor cells	Immun cells	
Case 1	63, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 2	67, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 3	31, Male	Positive	Positive	Negative	Positive	Positive	Positive	–	+	AWD
Case 4	52, Male	Positive	Positive	Negative	Positive	Positive	Positive	–	+	AWD
Case 5	62, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 6	57, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 7	41, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 8	57, Male	Positive	Positive	Negative	Positive	Positive	Positive	–	+	AWD
Case 9	65, Male	Positive	Positive	Negative	Positive	Positive	Positive	–	+	AWD
Case 10	47, Female		Positive	Negative	Positive	Positive	Positive	–	–	AWD

EMA – epithelial membrane antigen; PD-L1 – programmed death ligand 1; AWD – alive with disease

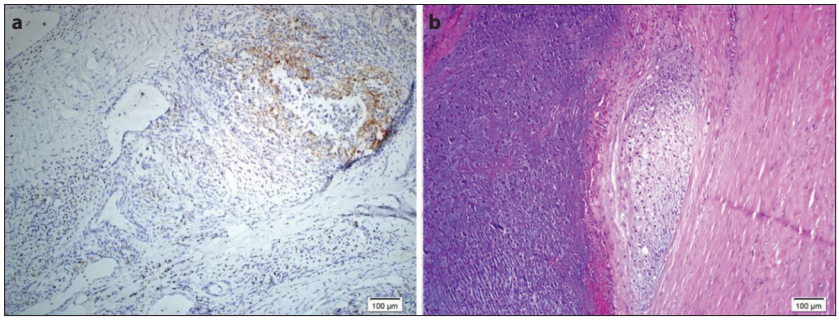

Figure 2. Immunohistochemical results of the tissue sample from the patient (case 2); this case had no recurrence; note that tumor cells and immune cells do not stain; soft tissue infiltration is also observed

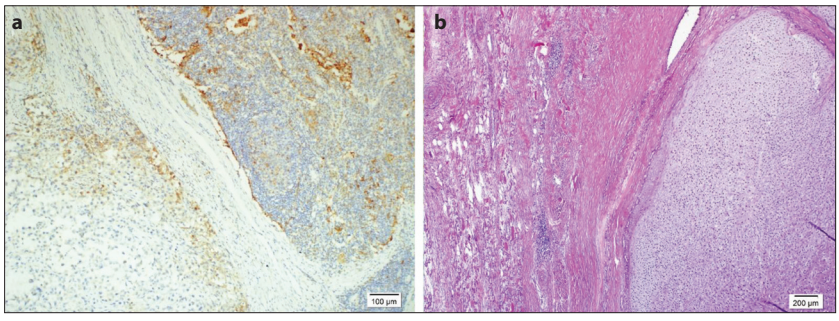

Figure 3. Immunohistochemical results of the tissue sample from the patient (case 3); this case had recurrence; tumor cells do not stain; however, tumor-infiltrating lymphocytes express more than 5 % programmed death ligand 1

There could be a delay in diagnosis, ranging from months to years. In our study, magnetic resonance imaging was used as the standard radiological tool for the evaluation of the tumor. Chest imaging revealed pulmonary metastasis. A biopsy confirmed the histological diagnosis. Magnetic resonance imaging resonance imaging revealed T1 isointense and T2 hyperintense enhancements. Despite aggressive surgery, cancer can metastasize after many years or recur, similar to that observed in the present study. In this clinical setting, subsequent surgery can result in higher morbidity. Therefore, there are limited options for medical therapy, with minimal efficacy [7].

The role of immunotherapy in soft tissue sarcomas is well known [8]. PD-L1 is one of the primary targets for immunotherapy. This study demonstrated that chordomas do express PD-Ll. Several studies have investigated the genetic basis of chordomas, and investigations on prognostic survival factors is ongoing, as demonstrated by a recent review of 78 genetic studies [9]. However, few studies have evaluated the role of PD-L1 in the pathogenesis of chordoma.

Feng et al. [10] investigated the expression score of PD-L1. Expression was higher in metastatic chordomas, with elevated levels of tumor-infiltrating

lymphocytes (TILs). Based on clinical trial data on chordoma treatment, they concluded that PD-L1 inhibition could be a possible immunotherapeutic strategy. Zou et al. [11] found that PD-L1 expression in TILs is associated with local recurrence and overall survival in spinal chordoma. In a more recent study, Zou et al. [12] developed an immunologic score, including CD3+, CD4+, CD8+, CD20+, Foxp3+, PD-1+, and PD-L1+ T cells. A significant correlation has been found between overall survival and the number of PD-1+ and PD-L1+ cells.

Chordoma, a rare bone tumor arising from the notochord, is resistant to conventional cancer treatments. The anti-PD-L1 drug, avelumab, was investigated as a potential treatment for chordoma. Fujii et al. [13] demonstrated killing of chordoma cells by natural killer-cell through antibody-dependent cell-mediated cytotoxicity of PD-L1-expressing tumor cells. Thus, they stated that the PD-1/PD-L1 pathway may be a novel therapeutic target for chordoma immunotherapy. Migliorini et al. [14] reported three cases of metastatic and locally advanced chordoma treated with different immunotherapeutic drugs. Two of them were administered with anti-PD-1 antibodies. Good clinical and radiological responses were observed. These findings suggest an immunogenic nature of chordomas.

Similar to our study, Mathios et al. [15] found that chordoma cells do not demonstrate significant PD-L1 expression; however, PD-L1 expression is evident in tumor-infiltrating macrophages and lymphocytes. Clinically, the role PD-L1 targeted therapy in recurrent chordomas was confirmed by Bishop et al. [16]. In these series, 15 out of 17 patients receiving immune checkpoint inhibitors had clinical benefit. This study supports the application of PD-L1 inhibition therapy in chordoma patients.

In literature, different staining rates have been accepted as positive expressions. This makes it difficult to interpret the results in terms of clinicopathological correlations. In our study, the cases with increased expression were > 5%. Therefore, we accepted 5% as the threshold. We believe that a linear correlation without using a threshold value is needed for the association between PD-L1 expression and clinicopathologic variables.

Study limitation

The main limitation of this study was the small sample size. Although recurrent cases have been found to have positive expression, the correlation of PD-L1 expression with recurrence, metastasis, and survival is needed from a larger population. Furthermore, it is not known how immunotherapy affects PD-L1 expression. Future research should evaluate the response rate of different therapeutic agents in PD-L1 positive cases.

CONCLUSION

In conclusion, this study demonstrated that PD-L1 expression is negative in nonrecurrent chordomas. These findings demonstrate less aggressive forms of chordomas, which do not require immunotherapy. Limitations arise from the limited number of chordoma cases considered in this study. Multicenter studies evaluating PD-L1 expression will have future implications for anti PD-L1 therapy, similar to other nonsurgical treatments.

Conflict of interest: None declared.

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Процена експресије *PD-L1* у рекурентним неметастатским сакралним хордомима – ретроспективна студија

Тулај Зенгинкинет[1], Ерхан Окај[2], Ајше Нур Токсоз Јилдирим[1], Ајкут Челик[2], Јалчин Турхан[3], Корхан Озкан[2], Мухлик Акурек[4]

[1]Градска болница Гозтепе „Проф. др Сулејман Јалчин", Одељење за патологију, Истанбул, Турска;
[2]Градска болница Гозтепе „Проф. др Сулејман Јалчин", Одељење за ортопедију, Истанбул, Турска;
[3]Универзитет у Дузџеу, Медицински факултет, Катедра за ортопедију и трауматологију, Дузџе, Турска;
[4]Болница „Марија-Јосеф" у Гревену, Клиника за ортопедију, Гревен, Оснабрук, Немачка

САЖЕТАК

Увод/Циљ Хордоми су ретки тумори нотохордалног порекла. За лечење се препоручује широка хируршка ресекција. Међутим, постоји високи ризик од морбидитета и морталитета. Поред тога, ови тумори су отпорни на хемотерапију. Дакле, за лечење хордома је потребна циљана терапија. Лиганд програмиране смрти 1 (*PD-L1*) обећавајући је избор за лечење рака. Истраживали смо експресију *PD-L1* код болесника са хордомом у студији једног центра.

Методе Блокови уграђени у парафин фиксирани у формалину процењени су за имунохистохемијску анализу да би се проценила експресија *PD-L1*. Клиничко-патолошке варијабле, као што су пол, старост и подаци о праћењу (понављање и исход), ретроспективно су прикупљени из медицинске документације болесника.

Резултати У ову студију је укључено десет болесника са дијагнозом сакралног хордома у једној установи између децембра 2015. и новембра 2021. године. Средња старост болесника је била 57 година, а средњи период праћења био је 40 месеци. Хируршке маргине су биле негативне у свим случајевима, без икаквог преоперативног медицинског третмана. Три од 10 болесника показала су позитивност *PD-L1* на имуним ћелијама. Ови болесници су показали локални рецидив, без метастаза. Средње време до локалног рецидива било је 15 месеци. Сви болесници су били живи на крају студије.

Закључак Ова студија је показала да се позитивност *PD-L1* у имуним ћелијама може користити као предиктивни маркер за локални рецидив у време хируршког лечења, што се може користити за утврђивање неопходности давања имунотерапије.

Кључне речи: хордом; широка хируршка ресекција; *PD-L1*; имунотерапија