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ORIGINAL RESEARCH

The Influence of Drug-Eluting Beads Transarterial Chemoembolization on Serum Levels of Soluble Programmed Cell Death Protein-1 in Advanced Hepatocellular Carcinoma Patients

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Aim: This study aims to explore the role of soluble programmed cell death protein 1 (sPD-1) in individuals with hepatocellular carcinoma (HCC) undergoing treatment with drug-eluting beads transarterial chemoembolization (D-TACE). Additionally, we aim to assess the potential utility of sPD-1 for determining the optimal timing for combining D-TACE with immune checkpoint inhibitors (ICIs).

Materials and Methods: A total of 44 HCC patients eligible for D-TACE and 55 healthy volunteers were enrolled in this study. Three milliliters of peripheral venous blood from the patients were collected on the day before D-TACE and 3, 7, and 30 days after D-TACE, respectively, for the assay of sPD-1. The relationships between sPD-1 levels, clinical features, outcomes, and the fluctuation of sPD-1 during treatment were analyzed.

Results: The initial sPD-1 levels in patients were found to be significantly higher than those in the control group. Although the initial sPD-1 levels displayed a decreasing trend with an increase in BCLC stage, no significant differences were observed among patients at different BCLC stages. The sPD-1 level on day 3 after D-TACE was similar to that on day 7 after D-TACE and significantly lower than the initial level. The sPD-1 level on day 30 after D-TACE was significantly higher than that on day 3 and day 7 after D-TACE and nearly returned to the initial level before D-TACE.

Conclusion: The level of sPD-1 was found to be significantly elevated in patients with HCC. However, further research is deemed necessary to fully understand the role of sPD-1 as a potential biomarker in the initiation, progression, and prognosis of HCC. The decrease in sPD-1 following D-TACE suggests that immune effector cells might potentially be reduced, as well as immune function weakened, highlighting the need to avoid the prompt administration of ICIs after D-TACE.

Keywords: hepatocellular carcinoma, immunotherapy, soluble programmed cell death protein 1, drug-eluting beads transarterial chemoembolization

Introduction

Hepatocellular carcinoma (HCC) stands as a significant contributor to global cancer-related mortality.[1] The majority of HCC patients are typically diagnosed at intermediate or advanced stages, making them ineligible for curative treatments.[2] Furthermore, HCC often exhibits a propensity for recurrence even following curative interventions.[3] Thus, there is a pressing need to discover more effective treatments for advanced-stage HCC, with the goal of extending patient survival. Presently, systemic therapies are commonly recommended for advanced-stage patients,[4] such as tyrosine kinase inhibitors (TKIs), immune checkpoint inhibitors (ICIs), and combinations of ICI and TKI.

Immunotherapy targeting programmed cell death protein-1 (PD-1), programmed cell death ligand-1 (PD-L1), and cytotoxic T-lymphocyte antigen-4 (CTLA-4) is rapidly advancing in the field of HCC.[5] An increasing body of evidence demonstrates the effectiveness of immunotherapy in the management of various malignancies.[6–9] However, the efficacy

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of ICI monotherapy remains limited in HCC.[10,11] Findings from the ImBrave150 study underscore the imperative need for combination therapies to enhance patient outcomes.[12] Besides the combination of ICIs and anti-vascular agents, multiple clinical trials are exploring the effectiveness of alternative combination strategies, including regional and systemic therapies, and preliminary results are encouraging.[13] Nonetheless, the challenge of identifying the optimal treatment for individual patients remains unresolved due to the absence of ideal biomarkers.

According to the 2022 Edition of China Liver Cancer Staging (CNLC), transarterial chemoembolization (TACE) is considered the primary treatment for intermediate and advanced stages of HCC. It has been substantiated to positively impact patient survival, supported by substantial data.[14] Theoretically, TACE exerts pleiotropic effects on modulating the tumor microenvironment, making it a potential candidate for combination with ICIs. The embolization agents employed in TACE can occlude the tumor's feeding arteries, inducing ischemic necrosis within the tumor.[15] This process may stimulate an acute inflammatory response and the release of tumor antigens, potentially enhancing the immune system's response, which had been previously suppressed. However, the precise impact of TACE on the immune response in HCC remains to be fully elucidated.

In this study, we aim to investigate the influence of D-TACE, one major kind of TACE, on the tumor microenvironment (TME) in HCC by analyzing the fluctuations in the level of soluble programmed cell death protein 1 (sPD-1) during the course of D-TACE.

Materials and Methods

Patient Selection

Between May 2019 and February 2022, HCC patients eligible for TACE were prospectively recruited. A total of 44 HCC patients were enrolled in this study, consisting of 36 males and 8 females, with an average age of 58 years (range: 40–81). Clinical diagnosis of HCC was carried out in accordance with the diagnostic criteria recommended in the Diagnostic and Treatment Practices for Hepatocellular Carcinoma (2019 edition, People's Republic of China). Inclusion criteria included an ECOG score of 0–2, Child-Pugh classification of A or B, measurable lesions, and no history of prior antitumor treatments. Exclusion criteria involved Child-Pugh classification C, the presence of significant arterio-portal or arterio-venous shunts, the existence of widespread metastases, and an estimated survival of less than 3 months. The control group was comprised of 55 healthy volunteers. Ethical approval for the study was obtained from our hospital's ethics committee, and informed consent was secured from all participants.

Clinical features, encompassing gender, age, Child-Pugh score, HBV DNA level, alpha-fetoprotein (AFP) level, Barcelona clinic liver cancer (BCLC) stage, baseline imaging characteristics such as the number of lesions, maximum tumor diameter, unilobar or bilobar distribution, vascular invasion, and therapeutic response based on mRECIST criteria, were documented.

Sampling and sPD-1 Measurement

Peripheral venous blood samples were collected from both HCC patients and healthy volunteers. For HCC patients, blood samples were acquired one day before and at 3, 7, and 30 days after D-TACE. Blood samples were drawn using Vacutainer tubes (BD Biosciences, NJ, USA) and were subsequently centrifuged at 3000 rpm for 5 minutes at 4°C. Following this, an additional 10-minute centrifugation was carried out to generate cell-free plasma, which was then immediately frozen at −80°C for subsequent analysis.

The serum sPD-1 level was determined using an enzyme-linked immunosorbent assay (ELISA) Kit (Abcam Plc, Cambridge, UK), in accordance with the manufacturer's instructions. The ELISA featured a detection limit of 9.6 pg/mL and had a detection range spanning from 25 to 1600 pg/mL.

D-TACE Procedure

All procedures were conducted using the GE3100 DSA system. Before the procedure, doxorubicin in the range of 60–80mg was loaded onto drug-eluting beads (CalliSpheres, Jiangsu Hengrui Medicine Co. Ltd., China). The size of the beads, ranging from 100–300 or 300–500μm, was selected based on the tumor diameter and blood supply characteristics. Initially, diagnostic angiographies were performed using a 4F RH catheter to gather all pertinent tumor information, including location, diameter,

and feeding arteries. Subsequently, a microcatheter was advanced in a superselective manner into the feeding artery. The drug-eluting beads were then slowly injected into the vessel under fluoroscopy. Upon reaching the intended endpoint, a follow-up angiography was performed to assess the effectiveness of embolization.

Statistical Analysis

Continuous variables were presented as mean±standard deviation, while categorical variables were expressed as rates. The *t*-test was employed to compare sPD-1 levels between different groups. All statistical tests were two-sided, and significance was defined as $P<0.05$. Data analysis was carried out using IBM SPSS software version 26.0.

Results

A total of 44 HCC patients were included in this study, comprising 36 males and 8 females, with an average age of 58 years (range: 40–81). The majority of patients were classified as having a Child–Pugh score of A (31/44, 70.5%), while the remaining 29.5% were classified as having a Child–Pugh score of B. Elevated AFP levels were observed in 61.4% (27/44) of patients, and 54.5% (24/44) exhibited a high level of HBV-DNA (≥500 IU/mL). Tumor size varied from 1.3 to 20 cm, with an average diameter of 8.86 cm. Unilobar tumors were present in 59.1% (26/44) of cases, and portal vein invasion was detected in 52.3% of patients (23/44). According to the BCLC staging, nine patients were classified as stage A (20.4%), eight patients as stage B (18.2%), and twenty-seven patients as stage C (61.4%). Detailed patient characteristics are displayed in Table 1. Furthermore, we conducted a correlation analysis between the initial sPD-1 level and

Table 1 Comparison of sPD-1 According to Clinical Characteristics of HCC Patients

	No.	Mean sPD-1	P value
Sex			0.421
Male	36	279.37±191.94	
Female	8	339.73±180.35	
Age			0.798
≥60yrs	15	280.00±162.35	
<60yrs	29	295.70±204.38	
BCLC stage			0.551
A stage	9	350.74±145.30	
B stage	8	291.46±136.55	
C stage	27	269.88±214.60	
Portal venous invasion			0.909
Yes	23	293.53±222.75	
No	21	286.86±149.82	
Child-Pugh score			0.846
A	31	286.70±182.52	
B	13	299.03±212.17	
AFP (ng/mL)			0.405
≤20	17	320.72±156.27	
>20	27	271.22±207.98	
HBV-DNA(IU/mL)			0.574
<500	20	308.18±149.87	
≥500	24	275.48±218.95	
Tumor location			0.958
Monolobar	26	291.62±182.31	
Bilobar	18	288.51±204.35	
Up to 7 criteria			0.191
≤7	9	364.44±184.78	
>7	35	271.29±188.32	

serum AST, ALT, and ALP levels using Spearman and Pearson correlation tests, but no statistically significant differences were found (P values were 0.642, 0.417, and 0.071, respectively).

Comparison of sPD-1 Level Between Patients and Control Group and Association of Initial sPD-1 Levels with Clinical Features

The sPD-1 level in the 44 patients was significantly higher than in the control group (290.34 ± 189.31 pg/mL vs 221.26 ± 94.35 pg/mL, P = 0.031; see Figure 1). Although the sPD-1 level exhibited a decreasing trend with an increase in BCLC stage, no significant differences were observed (BCLC stage A, B, C: 350.74 ± 145.30, 291.46 ± 136.55, 269.88 ± 214.60, P = 0.551; see Figure 2). No other significant associations were identified between the sPD-1 level and other clinical factors, including age, sex, Child-Pugh score, portal vein invasion, up to seven criteria, AFP level, and HBV-DNA(see Table 1).

Fluctuation of the sPD-1 Level During the Session of TACE Treatment

The fluctuation of sPD-1 levels in seven HCC patients during the course of D-TACE treatment was examined. The sPD-1 level at 3 days post-D-TACE was 112.48 ± 91.91 pg/mL, which was significantly lower than before D-TACE (P = 0.032; see Figure 3). The sPD-1 level at 7 days post-D-TACE was 123.32 ± 100.96 pg/mL, indicating a slight increase compared to 3 days post-D-TACE (P = 0.541), but it remained lower than the initial sPD-1 level (P = 0.059), with no significant differences observed. Subsequently, the sPD-1 level exhibited an upward trend, and at 30 days post-D-TACE, the sPD-1 level was 174.45 ± 116.35 pg/mL, significantly higher than at 7 days post-D-TACE(P = 0.002) and nearly returning to the pre-D-TACE level (P = 0.920).

The influence of callispheres with different diameters was also investigated. There were no significant differences in sPD-1 levels between 100–300μm and 300–500μm beads at 3 days post- D-TACE (260.24 ± 167.78 vs 253.70 ± 181.15, P = 0.914; see Figure 4). However, with respect to 7 days post-D-TACE, significant differences were observed (310.86 ± 127.91 vs 158.66 ± 107.06, P = 0.017; see Figure 5).

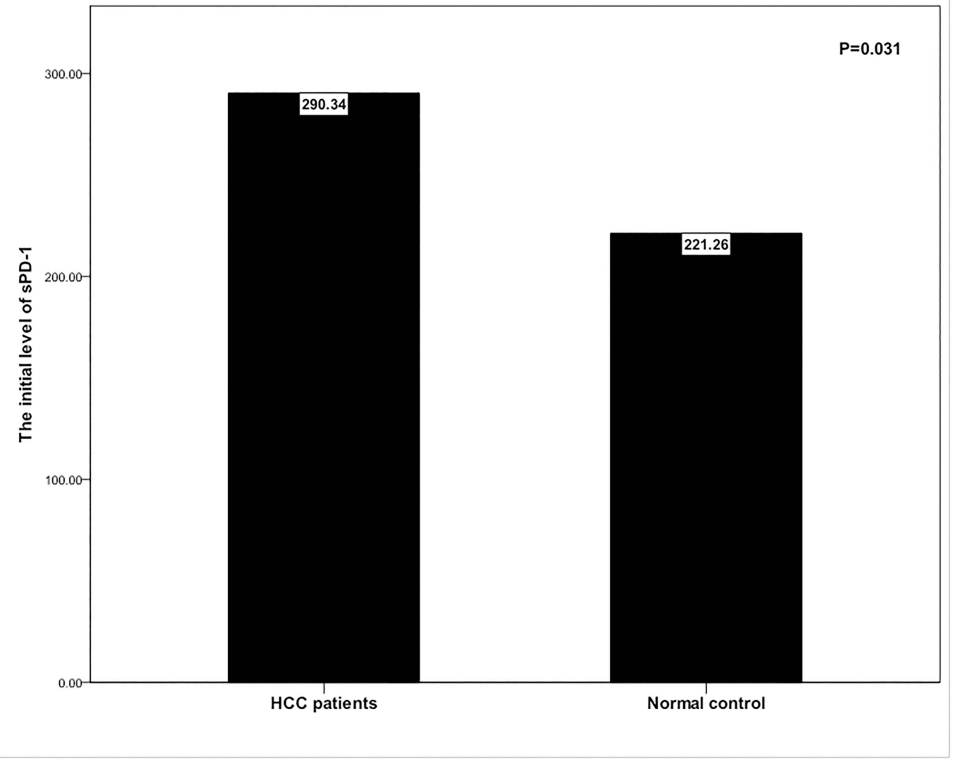

Figure 1 Comparison of the initial sPD-1 levels between HCC patients and normal controls. A significant difference was observed between the two groups (P = 0.031).

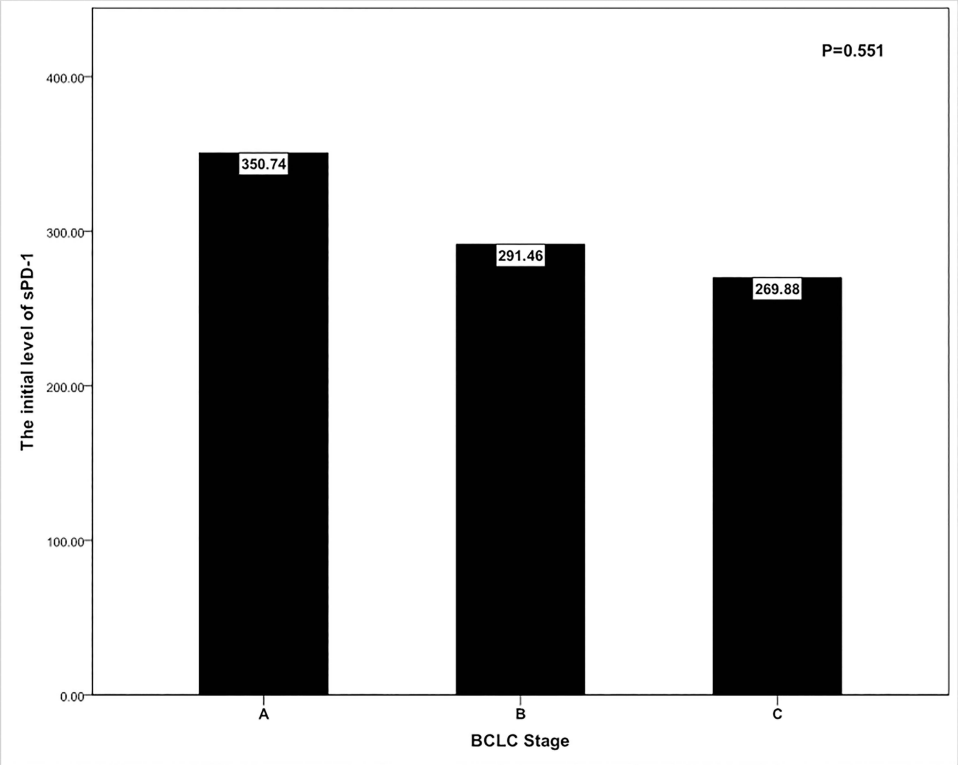

Figure 2 Comparison of the initial sPD-L1 levels among different BCLC staging categories. No significant differences were detected (P = 0.551).

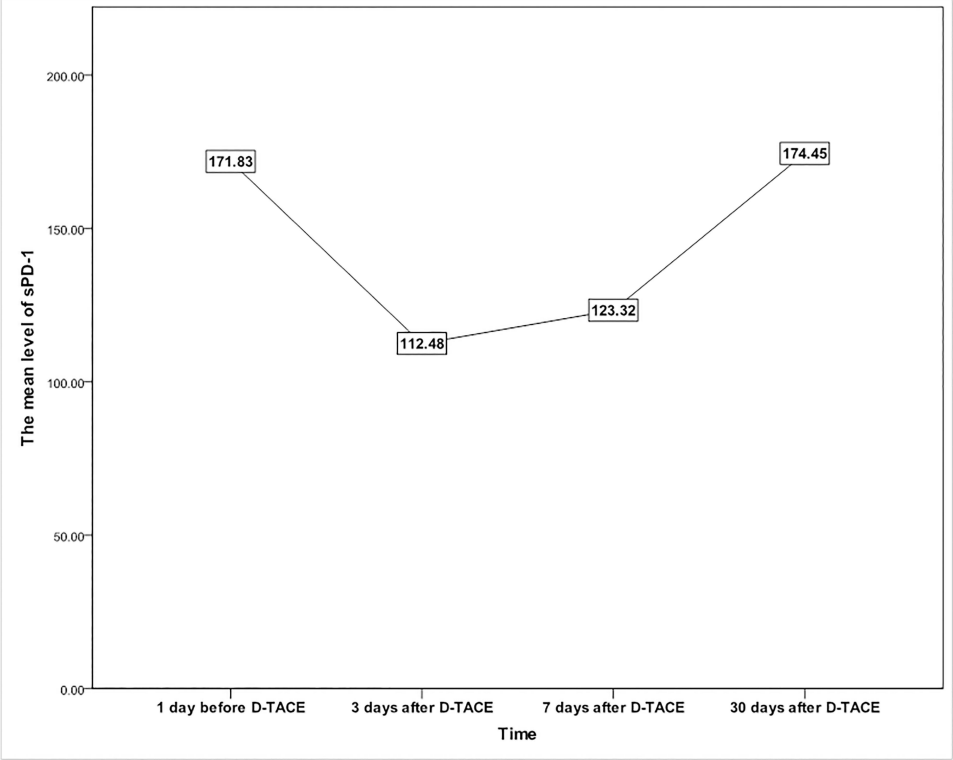

Figure 3 Fluctuation of sPD-1 levels in seven HCC patients during the course of D-TACE treatment. The sPD-1 level on 3 days post-D-TACE was lower than that before D-TACE (P = 0.032), and the sPD-1 level on 30 days post-D-TACE was higher than that on 3 and 7 days post-D-TACE (P = 0.039, P = 0.002). However, there were no significant differences between sPD-1 levels on 3 and 7 days post-D-TACE (P = 0.541) and between 1 day before D-TACE and 30 days after D-TACE (P = 0.920).

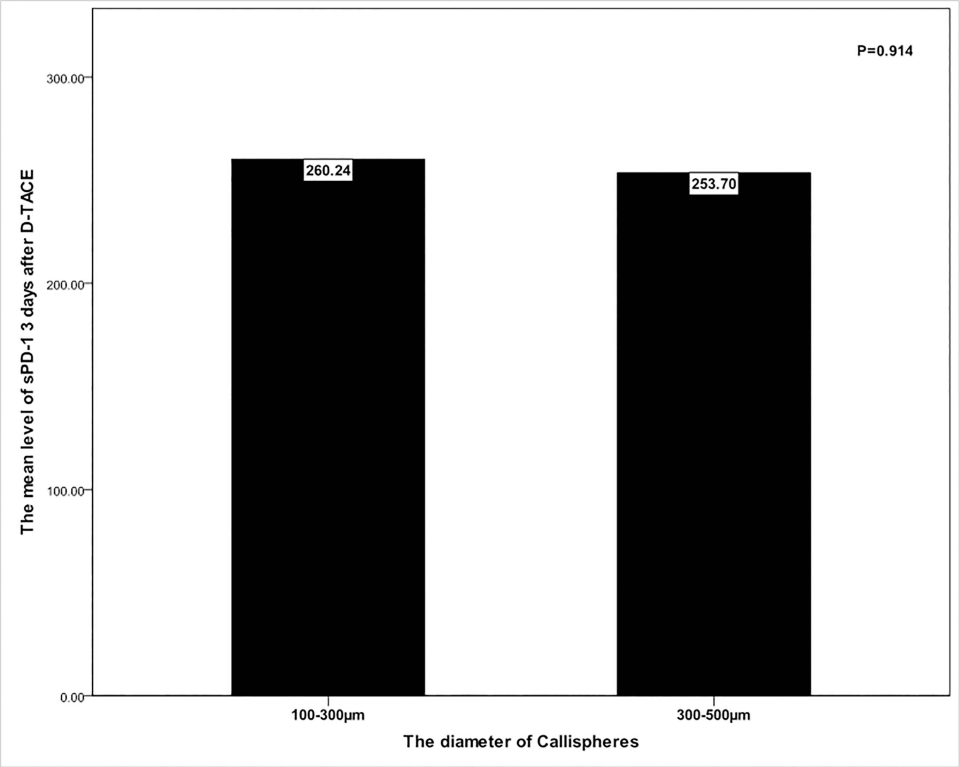

Figure 4 Comparison of sPD-1 levels on 3 days after D-TACE between 100–300μm and 300–500μm Callispheres beads. No significant differences were observed (P = 0.914).

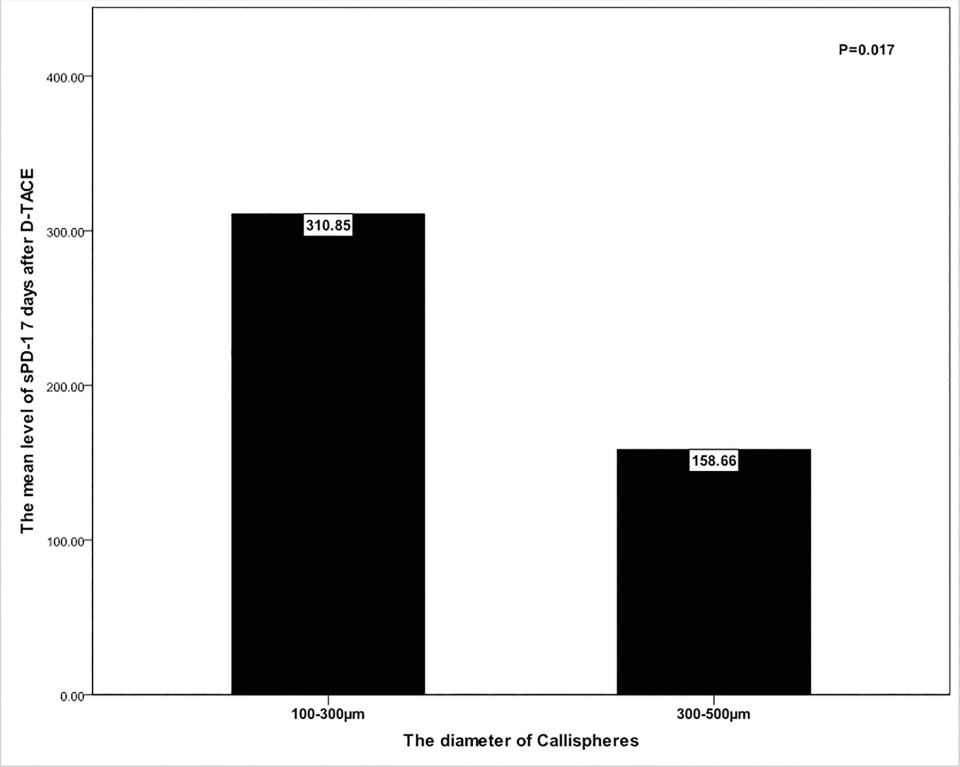

Figure 5 Comparison of sPD-1 levels on 7 days after D-TACE between 100–300μm and 300–500μm Callispheres beads. The results showed significant differences (P = 0.017).

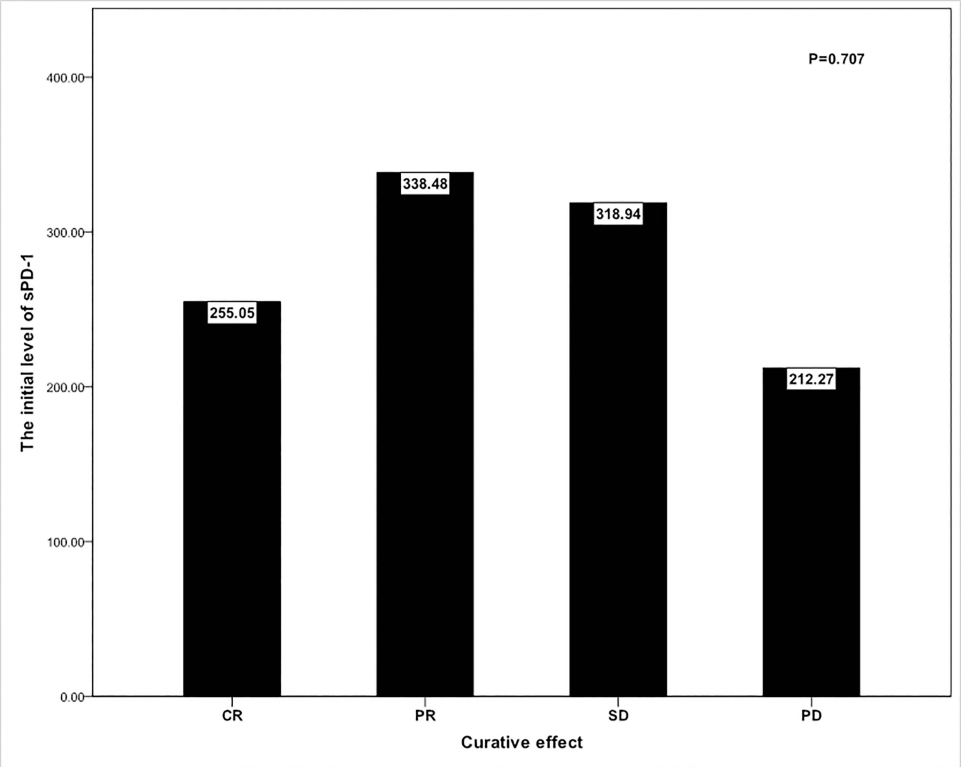

Figure 6 Comparison of the initial sPD-1 levels among different curative effect groups 30 days after D-TACE based on mRECIST criteria. No significant differences were found (P = 0.707).

The one-month tumoral response to D-TACE was evaluated in 35 patients according to mRECIST criteria. The analysis revealed 3 complete responses (CR), 8 partial responses (PR), 20 stable diseases (SD), and 4 progressive diseases (PD). The sPD-1 level one day before D-TACE in CR and PD patients was lower than in PR and SD patients, but the differences were not statistically significant (P = 0.707; see Figure 6).

Discussion

HCC stands as a prominent contributor to cancer-related mortality on a global scale. Despite the promising results demonstrated by systemic treatments in other types of cancer, a significant proportion of advanced HCC patients exhibit inadequate responses to these therapies. Combining local and systemic treatment strategies holds substantial potential to enhance patient outcomes. Although there are several clinical trials examining the effectiveness of such combination approaches, only a limited number have explored the potential impact of Transarterial Chemoembolization (TACE) on the immune profiles of HCC from the perspective of soluble molecules, such as the expression of sPD-L1 and sPD-1. Even fewer studies have focused on the early alterations in the immune microenvironment following TACE.[16–19]

Notably, our observations revealed a marked decrease in sPD-1 expression three days after D-TACE. The activation of the PD-1/PD-L1 pathway is one of the most critical mechanisms for tumor immune evasion, involving the inhibition of T-cell proliferation, induction of T-cell exhaustion, and enhancement of regulatory T cell activity.[20] Within the PD-1/PD-L1 pathway, there are two types of molecules: membranous form (mPD-1/mPD-L1) and soluble form (sPD-1/sPD-L1). Both types of molecules play crucial roles in the tumor immune response, but their specific functions differ.[21,22] Membrane-form molecules mediate costimulatory signals through direct receptor-ligand interactions, while soluble-form molecules can exert their influence on nearby as well as distant cells by binding to receptors on their surfaces. Consequently, soluble molecules may have a more significant role in the onset and progression of diseases.[23] Recent research has indicated that the expression of membranous molecules is correlated with tumor staging and prognosis. Some studies have suggested that these molecules could serve as potential biomarkers for guiding Immune Checkpoint Inhibitors (ICIs) therapy.[24,25] However, in clinical

practice, a substantial proportion of HCC patients are already in an advanced stage at the time of their initial diagnosis. As a result, they are not suitable candidates for radical therapies, making it impractical to obtain tumor tissue for the analysis of mPD-1/mPD-L1 expression. Conversely, assessing sPD-1/sPD-L1 expression in peripheral venous blood is a more convenient and less invasive approach. Additionally, peripheral venous blood can be repeatedly sampled to dynamically monitor changes in sPD-1/sPD-L1 expression throughout the entire treatment procedure.

Our study revealed a significant elevation in sPD-1 levels among HCC patients when compared to the control group. These findings align with previous research that has indicated an association between sPD-1 and the risk of HCC.[26] While our study showed a decrease in sPD-1 levels with an increase in BCLC stage, these changes did not reach statistical significance. Furthermore, we found no significant associations between sPD-1 levels and portal vein invasion or the up-to-seven criteria. Further investigations are warranted to assess the potential of sPD-1 in predicting the onset, development, and prognosis of HCC.

The chemotherapeutic agents employed in TACE, along with the embolization of tumor-feeding arteries, can induce local inflammation and tumor necrosis. The breakdown of tumor cells can lead to the release of tumor antigens, which can be taken up by antigen-presenting cells (APCs), subsequently eliciting tumor-associated antigen-specific responses.[27,28] This is believed to have a positive impact on ICIs therapy. However, TACE can induce rapid hypoxia in the tumor microenvironment, giving rise to various hypoxia-related factors that can swiftly influence the components of cancer immunity.[17] There are limited studies examining the early effects of TACE on sPD-1 levels in advanced HCC patients. We observed that sPD-1 levels at 3 and 7 days after D-TACE were significantly lower than those prior to the procedure. However, the sPD-1 level at 30 days after D-TACE was notably higher than at 7 days post-D-TACE and nearly returned to the initial pre-D-TACE level. As is widely known, PD-1 is predominantly expressed in activated CD8+, CD4+ lymphocytes, and NK cells.[29,30] sPD-1 can result from the cleavage of their extracellular domains or from alternative splicing of the pre-mRNA coding for the membrane form,[31] thereby partially reflecting the expression of mPD-1. Consequently, our findings suggest that D-TACE may lead to a reduction in the level of immune effector cells over a short time period. Prior studies have shown that 1–2 weeks following Gelatin Sponge Microparticles TACE (GSMs-TACE), CD8+ T cells were significantly lower than before GSMs-TACE.[32] Additionally, Doxorubicin, the most frequently used chemotherapy drug in TACE, can induce the death of immunogenic cells.[33] Thus, the diminished presence of immune effector cells implies that, at least within the first week after TACE, initiating ICIs therapy may not be optimal. Some studies have also indicated that sPD-1 can serve as a blockade for PD-1/PD-L1 interactions to restore suppressed immune responses.[34,35] However, the reduction in sPD-1 levels one week after TACE may weaken this effect, offering further evidence that initiating ICIs shortly after TACE may be less effective.

While the combination therapy of TACE and ICIs has been investigated in numerous studies,[28] there remain several unresolved issues. These include the determination of the optimal timing for commencing ICIs administration, a comprehensive understanding of how TACE impacts the immunological microenvironment, and further investigations to determine the preferred choice of embolic agents to combine with ICIs.

This study has notable limitations. First, the sample size is restricted. Second, the intervals for blood sampling were inadequate, which limited our ability to examine the fluctuations in sPD-1 levels between 7 and 30 days following D-TACE.

In summary, the study highlights that sPD-1 levels were significantly elevated in HCC patients. However, additional research is imperative to delve into the potential of sPD-1 as a predictive marker for the onset, progression, and prognosis of HCC. It is evident that D-TACE might reduce immune effector cells and impair immune function, suggesting that ICIs should not be administered shortly after D-TACE.

Data Sharing Statement

All data generated or analysed during this study are included in this published article.

Ethical Statement

Therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki (Revised in 2013). This study approved by the Medical Ethics Committee of Qilu Hospital of Shandong University [(C)

Review No. 2018 (140)], and informed consent was secured from all participants. All methods were carried out in accordance with relevant guidelines and regulations.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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