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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Research Article

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Abstract

Aims: To investigate the implications of soluble programmed cell death protein 1 (sPD-1) in hepatocellular carcinoma (HCC) patients treated with drug-eluting beads transarterial chemoembolization (D-TACE) and to evaluate the potential value of sPD-1 to guide selection of the optimal time to begin combination therapy with D-TACE and immune checkpoint inhibitors (ICIs).

Materials and methods: Forty-four HCC patients suitable for TACE and fifty-five healthy volunteers were enrolled in this study. Three milliliters of peripheral venous blood of patients were collected on 1 day before TACE and 3, 7, and 30 days after TACE respectively for assay of sPD-1 using enzyme-linked immunosorbent assay. The associations of the sPD-1 level with the clinical features, outcomes, and the fluctuation of sPD-1 during the treatment were analyzed.

Results: The initial sPD-1 level of patients was significantly higher than that of the control group. Although the initial level of sPD-1 showed a decreasing trend with the increase of BCLC stage, there were no significant differences among patients with different BCLC stages. The sPD-1 level of 3 days after TACE was significantly lower than the initial level but the level of sPD-1 after 7 days of TACE was similar to that after 3 days of TACE. The sPD-1 level of 30 days after TACE was significantly higher than that of 7 days after TACE. When it came to 30 days after TACE, sPD-1 level nearly elevated to the initial level before TACE. The level of sPD-1 of CR and PD patients was lower than that of PR, SD patients, but the differences were not significant.

Conclusion: The level of sPD-1 was significantly elevated in patients with HCC but further research is necessary to better understand the value of sPD-1 in onset, development, and prognosis of HCC as a potential biomarker. The decreases in sPD-1 after D-TACE suggested that D-TACE could probably reduce immune effector cells as well as weaken immune function, which indicated that the ICIs shouldn't be administered shortly after D-TACE.


Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer deaths globally (1). Usually, a large part of HCC patients is diagnosed in the intermediate or advanced stages and are not candidates for curative treatments (2). What is worse, HCC is easy to recur even after curative treatments (3). It is crucial to find more effective treatment for advanced stage HCC to prolong patients' survival. Nowadays systemic therapies are indicated for those patients at advanced stages normally (4), such as tyrosine kinase inhibitors (TKIs), immune checkpoint inhibitors (ICIs) and combination of ICI and TKI.
The ICIs therapy targeting programmed cell death protein-1 (PD-1), programmed cell death ligand-1 (PD-L1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) are progressing rapidly in HCC (5). Nowadays more and more studies showed that immunotherapy was effective in the treatment of many kinds of malignancies (6–9). However, the efficacy of ICIs monotherapy is limited in HCC (10, 11). Results of the ImBrave150 study emphasize the necessity of combined treatments to improve patient outcomes (12). In addition to combination of ICIs and anti-vascular agents, there are many clinical trials to explore the efficacy of other combination strategy, such as regional and systemic therapies and the preliminary results are promising (13). But the identification of the optimal treatment for a specific patient is still an unanswered question because of lacking the ideal biomarkers.

According to 2022 Edition of China Liver Cancer Staging (CNLC), transarterial chemoembolization (TACE) is the first-line treatment for intermediate and advanced stages of HCC. It has been demonstrated to have a positive effect on improving survivals and the efficacy has been supported by large cohorts (14). Theoretically, TACE has the pleiotropic effects on modulating the tumoral microenvironment which may be suitable for combination with ICIs. Embolization agents can obstruct the tumor feeding arteries, resulting in ischemic necrosis of the tumor (15). It is possible that the acute inflammation and the liberation of antigens caused by the necrosis greatly enhance the response of immune system which was previously inhibited. However, the immune response affected by TACE to HCC remain to be fully elucidated.

In this study we explored the influence of TACE on tumoral microenvironment (TME) of HCC by analyzing the fluctuation of the soluble programmed cell death protein 1 (sPD-1) level during the period of TACE.

Materials And Methods

2.1 Patient selection

HCC patients who were candidates of TACE were prospectively recruited between May 2019 and February 2022. The HCC was clinically diagnosed according to the diagnostic criteria recommended in the Diagnostic and Treatment Practices for Hepatocellular Carcinoma (2019 edition, People's Republic of China). The inclusion criteria included an ECOG score 0–2, Child–Pugh's level A or B, measurable lesions and no history of other antitumor treatments. The exclusion criteria included Child-Pugh's level C, significant arterio-portal or arterio-venous shunts, widespread metastases, estimated survival of less than 3 months. The control group was composed of fifty-five healthy volunteers. The study was approved by the ethics committee of our hospital. Informed consents were obtained from all the participants.

Clinical features including sex, age, Child-Pugh score, HBV DNA level, alpha-fetoprotein (AFP) level, Barcelona clinic liver cancer (BCLC) stage, baseline imaging features such as numbers of foci, maximum diameter of tumor, unilobar or bilobar lesion, and vascular invasion and the curative effect based on mRECIST criteria were collected.
2.2 Sampling And Spd-1 Measurement

Peripheral venous blood samples were obtained from healthy volunteers. The samples of patients were obtained on 1 day before and 3, 7, and 30 days after TACE. Blood samples were collected in Vacutainer tubes (BD Biosciences, NJ, USA) and were centrifuged at 3,000 rpm for 5min at 4°C. Additional 10min centrifugation was performed to produce cell-free plasma, after which the plasma were immediately frozen at −80°C for further analysis.

Using an enzyme linked immunosorbent assay (ELISA) Kit(Abcam Plc, Cambridge, UK), serum sPD-1 level was determined according to the instruction of the manufacturer. The detection limit for ELISA was 9.6pg/ml. And detection range was between 25 to 1600 pg/ml.

2.3 Deb-tace Procedure

Using GE3100 DSA system all the procedures were performed. 60-80mg doxorubicin was loaded to drug eluting beads (CalliSpheres, Jiangsu Hengrui Medicine Co. Ltd., China) before the procedure. The size of the beads was 100–300 or 300–500µm according to the tumor diameter and features of blood supply. Diagnostic angiographies were performed using a 4F RH catheter firstly. After getting all of the tumoral information such as location, diameter and feeding arteries, a microcatheter was superselectively advanced into the feeding artery. Then, the beads was injected slowly into the vessel under fluoroscopy. After the ending point was got, another round of angiography was performed to evaluate the efficacy of embolization.

2.4 Statistical Analysis

Continuous variables were shown as mean ± standard deviation and categorical variables were presented as rates. The t test was used to compare the sPD-1 levels of different groups. All statistical tests were two-sided. The significance was defined as P < 0.05. IBM SPSS software version 26.0 was used for data analysis.

Results

Forty-four HCC patients were enrolled in this study, including 36 males and 8 females with an average age of 58 (range 40–81) years. Most patients were classified as Child–Pugh score A (31/44, 70.5%) and the remaining 29.5% were classified as Child–Pugh score B. 61.4% (27/44) had an elevated AFP and 54.5% (24/44) had a high level of HBV-DNA (≥ 500 IU/ml). The maximum diameter of tumor ranged from 1.3 to 20 cm with an average of 8.86cm. Patients with unilobar tumors accounted for 59.1% (26/44). Portal vein invasion was found in 52.3% of the group (23/44). Nine patients were classified as BCLC stage A (20.4%). Eight patients were classified as BCLC stage B (18.2%). Twenty-seven patients were classified as BCLC C (61.4%). The detailed characteristics of the patients are shown in Table 1.
<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Mean sPD-1</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.421</td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>279.37 ± 191.94</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>339.73 ± 180.35</td>
<td></td>
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<tr>
<td>Age</td>
<td></td>
<td></td>
<td>0.798</td>
</tr>
<tr>
<td>≥ 60yrs</td>
<td>15</td>
<td>280.00 ± 162.35</td>
<td></td>
</tr>
<tr>
<td>&lt;60yrs</td>
<td>29</td>
<td>295.70 ± 204.38</td>
<td></td>
</tr>
<tr>
<td>BCLC stage</td>
<td></td>
<td></td>
<td>0.551</td>
</tr>
<tr>
<td>A stage</td>
<td>9</td>
<td>350.74 ± 145.30</td>
<td></td>
</tr>
<tr>
<td>B stage</td>
<td>8</td>
<td>291.46 ± 136.55</td>
<td>0.909</td>
</tr>
<tr>
<td>C stage</td>
<td>27</td>
<td>269.88 ± 214.60</td>
<td></td>
</tr>
<tr>
<td>Portal venous invasion</td>
<td>23</td>
<td>293.53 ± 222.75</td>
<td></td>
</tr>
<tr>
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<td>21</td>
<td>286.86 ± 149.82</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Child-Pugh score</td>
<td></td>
<td></td>
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<tr>
<td>A</td>
<td>31</td>
<td>286.70 ± 182.52</td>
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<tr>
<td>B</td>
<td>13</td>
<td>299.03 ± 212.17</td>
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<tr>
<td>AFP (ng/ml)</td>
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<td>0.405</td>
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<td>≤ 20</td>
<td>17</td>
<td>320.72 ± 156.27</td>
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<td>&gt;20</td>
<td>27</td>
<td>271.22 ± 207.98</td>
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<tr>
<td>HBV-DNA(IU/ml)</td>
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<td>0.574</td>
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<tr>
<td>&lt;500</td>
<td>20</td>
<td>308.18 ± 149.87</td>
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<tr>
<td>≥ 500</td>
<td>24</td>
<td>275.48 ± 218.95</td>
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</tr>
<tr>
<td></td>
<td>No.</td>
<td>Mean sPD-1</td>
<td>P Value</td>
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<tr>
<td>----------------------</td>
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<td>---------</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Monolobar</td>
<td>26</td>
<td>291.62 ± 182.31</td>
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<tr>
<td>Bilobar</td>
<td>18</td>
<td>288.51 ± 204.35</td>
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<tr>
<td>Up to 7 criteria</td>
<td>35</td>
<td>271.29 ± 188.32</td>
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<tr>
<td>≤ 7</td>
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<tr>
<td>&gt;7</td>
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3.1 Comparison of sPD-1 level between patients and control group and association of initial sPD-1 levels with clinical features

The sPD-1 level of 44 patients was significantly higher than the control group (290.34 ± 189.31pg/ml vs 221.26 ± 94.35pg/ml, P = 0.031; Fig. 1). Although the level of sPD-1 showed a decreasing trend with the increasing of BCLC stage, there were no significant difference (BCLC stage A, B, C: 350.74 ± 145.30, 291.46 ± 136.55, 269.88 ± 214.60, P = 0.551; Fig. 2). There were no other significant relationships between the sPD-1 level and other clinical factors, including age, sex, Child-Pugh’s score, portal vein invasion, up to seven criteria, AFP level and HBV-DNA.

3.2 Fluctuation of the sPD-1 level during the session of TACE treatment

The fluctuation of sPD-1 level of seven HCC patients during the treatment period of TACE was explored. The sPD-1 level of 3 days after TACE was 112.48 ± 91.91pg/ml, which was significantly lower than that before TACE (P = 0.032, Fig. 3). The sPD-1 level of 7 days after TACE was 123.32 ± 100.96pg/ml, which showed a slightly increasing compared to that of 3 days after TACE but still lower than the initial level of sPD-1. The differences were not significant (P = 0.541; P = 0.059). Then the level of sPD-1 showed an increasing trend and on 30 days after TACE the sPD-1 level was 174.45 ± 116.35pg/ml, which was higher than that of 7 days after TACE significantly (P = 0.002) and nearly recovered to the level before TACE (P = 0.920).

The influence on sPD-1 of callispheres with different diameter was also studied. There were no significant differences between 100–300µm and 300–500µm beads in the sPD-1 level 3 days after TACE (260.24 ± 167.78 vs. 253.70 ± 181.15, P = 0.914). However, concerning about 7 days after TACE, the result had significant differences (310.86 ± 127.91 vs. 158.66 ± 107.06, P = 0.017).

The one-month tumoral response to DEB-TACE of 35 patients was analyzed according to mRECIST criteria. There were 3 CR, 8 PR, 20 SD, and 4 PD patients. The sPD-1 level of 1 day before TACE in CR and PD patients was lower than that of PR, SD patients, but the differences were not significant (P = 0.707; Figs. 4).
Discussion

HCC is one of the leading causes of tumor deaths worldwide. Despite systemic treatments which have showed promising effect in other tumors are wildly used in patients of the advanced HCC, a substantial part of patients responds poorly to these treatments. The combination of local and systemic therapy may have tremendous talent to improve the patient’s outcome. Although, there are some clinical trials evaluating the effectiveness of such kind of combination, only a few have investigated the potential influence of TACE on HCC immune profiles from a soluble molecule point of view, such as the expression of sPD-L1 and sPD-1. Even fewer studies focused on the early changes of immune microenvironment after TACE (16–18). Interestingly, we observed a significantly lower sPD-1 expression in 3 days after TACE. Activation of PD-1/PD-L1 pathway was one of the most critical mechanisms of tumor evasion, inhibiting T-cell proliferation, inducing T-cell exhaustion, and enhancing the activity of regulatory T cells (19). There are two forms of molecules in PD-1/PD-L1 pathway: membranous form (mPD-1/mPD-L1) and soluble form (sPD-1/sPD-L1). The two kinds of molecules both play important roles in tumor immune response, but their specific roles are different (20, 21). Membrane-form molecules mediate costimulatory signals through direct receptor–ligand interactions while soluble-form molecules can affect near-end as well as far-end cells by binding receptors on their surfaces. So the soluble molecules may play a far more important role than membranous molecules in the occurrence and development of diseases (22). Recent findings have shown that expression of membranous molecules was correlated with staging of tumor, prognosis. And some researches showed that it could be a potential biomarker to guide ICIs therapy (23, 24). But in clinical application, a large part of HCC patients is in advanced stage in the first diagnosis. And they are not candidates to receive radical therapies. So, it is impractical to acquire the tumor tissue for analyzing mPD-1/mPD-L1 expression. While, it is easier and less traumatic to test sPD-1/sPD-L1 expression in the peripheral venous blood. Furthermore, the peripheral venous blood could be sampled repeatedly to dynamically monitor the changing of sPD-1/sPD-L1 expression during the whole treatment procedure.

Our study showed that sPD-1 level was significantly elevated in HCC patients compared to control group. These results are consistent with the previous findings showing that sPD-1 is associated with risks of HCC (25). Although our study showed that the sPD-1 level decreased with the increasing of BCLC stage, the results showed no significant differences among them. And there were no significant relationships between sPD-1 level and portal vein invasion, up to seven criteria. Further research is necessary to explore the value of sPD-1 in predicting the onset, development, and prognosis of HCC.

The chemotherapeutic drugs used in TACE and embolization of tumoral feeding artery could cause local inflammation and necrosis of the tumor. The disintegration of tumor cells could release tumor antigens, which could be taken up by APCs, then arouse tumor-associated antigen-specific responses (26, 27), which was thought to be a positive influence on ICIs therapy. However, TACE can induce sudden hypoxia in tumor microenvironment and produce numerous hypoxia-related factors, which can affect the components of cancer-immunity in a short time (17). There are few studies that explored the early influence of TACE on sPD-1 in advanced HCC patients. We found that the sPD-1 levels 3 and 7 days after
TACE were both much lower than that before TACE. However, the sPD-1 level of 30 days after TACE was significantly higher than that 7 days after TACE and nearly elevated to the initial level before TACE. As we all know, PD-1 are mainly expressed in activated CD8+, CD4 + lymphocytes and NK cells (28, 29). sPD-1 could be produced from the cleavage of their extracellular domains or from alternative splicing of the pre-mRNA coding for the membrane form (30), it could partially reflect the expression of mPD-1. So, our finding suggests that TACE could decrease the level of immune effector cells in a short period of time. Previous studies showed that 1–2 weeks after Gelatin Sponge Microparticles TACE(GSMs-TACE), the CD8 + T cells were significantly lower than that before the GSMs-TACE (31) and Doxorubicin which is the most used chemotherapy drugs in TACE can induce the death of immunogenic cells (32). So, the low level of immune effector cells suggests that at least within 1 week after TACE is not suitable for ICIs therapy. Some studies also found that sPD-1 could be used as a blockade of PD-1/PD-L1 interactions to restore the inhibited immune response (33, 34). While the decreasing of sPD-1 1 week after TACE may weaken this effect, it is another clue that suggests it may be less effective to begin the administration of ICIs early after TACE.

Although the combination therapy of TACE and ICIs have been explored in many studies (27), there are still many unsolved problems. We don’t know the optimal time to begin ICIs administration. The mechanism how TACE impacts the immunological microenvironment has not been fully explained. And which kind of embolic agents is a better choice to combine with ICIs also needs further investigation.

There were obvious limitations in the study. First, the sample size of this study is limited. Second, the time points of blood sampling were not sufficient, which let us miss the opportunity to study the fluctuation of sPD-1 between 7 and 30 days after TACE. In conclusion, sPD-1 level was significantly elevated in patients with HCC but further research is necessary to explore the value of sPD-1 in predicting the onset, development, and prognosis of HCC. TACE could probably reduce immune effector cells as well as weaken immune function, which suggests that the ICIs shouldn’t be administrated shortly after TACE.

Declarations

Ethics approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of Qilu Hospital of Shandong University (No. 2018(140)). All persons gave their informed consent prior to their inclusion in the study.

Consent for publication: All authors approved the final manuscript and the submission to this journal.

Availability of data and materials: The datasets during and analysed during the current study available from the corresponding author on reasonable request.

Competing Interests: No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.
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Authors’ contributions: KZ contributed to the conceptualization of this study and was a major contributor in writing the manuscript. KZ, XS and FX performed the experiment. XM, QW and WJ contributed significantly to analysis and manuscript preparation. KZ and XM performed the data analyses and wrote the manuscript. YX and CL helped perform the analysis with constructive discussions. All authors read and approved the final manuscript.

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Figures
Figure 1

Comparison of the initial level of sPD-1 between HCC patients and normal control. There was significant difference between the two groups (P=0.031)
Figure 2

Comparison of the initial level of sPD-L1 among different BCLC staging. There were no significant differences (P=0.551).
Figure 3

The fluctuation of sPD-1 level of HCC patients of seven patients during the treatment session of TACE. The sPD-1 level of 3 days after TACE was lower than that before TACE (P=0.032), and the sPD-1 level of 30 days after TACE was higher than that of 3 and 7 days after TACE (P=0.039, P=0.002), whereas there were no significantly differences between 7 and 3 days after TACE (P = 0.541) and between 1 day before and 30 days after TACE (P=0.920).
Figure 4

Comparison of the initial level of sPD-1 among the different curative effects groups 30 days after TACE based on mRECIST criteria. There were no significant differences (p = 0.707)