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Original Article

Efficacy of ifosfamide combined with liposome doxorubicin on osteosarcoma and its effects on serum IL-10, TNF-α, and IFN-γ in patients with osteosarcoma

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Abstract: Objective: To evaluate the effects of ifosfamide combined with liposome doxorubicin on osteosarcoma (OS) and its effects on serum IL-10, TNF-α, and IFN-γ in patients with OS. Methods: A total of 86 patients with OS who received chemotherapy in Honghui Hospital, Xi'an Jiaotong University from Jan. 2017 to Dec. 2019 were enrolled. Patients treated by conventional doxorubicin + ifosfamide were assigned to the regular group (n=40). Others treated by liposome doxorubicin + ifosfamide were assigned to the research group (n=46). The clinical efficacy, 2-year survival rate, and adverse reactions of the two groups were evaluated and compared. ELISA was adopted for quantification of tumor specific growth factor (TSGF), vascular endothelial growth factor (VEGF), erb-b2 receptor tyrosine kinase 3 (ERBB3), tumor necrosis factor-α (TNF-α), interferon-gamma-γ (IFN-γ), and interleukin-10 (IL-10). The EORTC Quality of Life Questionnaire (QLQ-C30) was adopted to evaluate a patient's life quality. Results: The research group showed a higher total effective rate and a higher 2-year survival rate than the regular group, but lower incidences of liver and kidney function injury, thrombocytopenia, and cardiotoxicity than the regular group. After therapy, lower levels of serum TSGF, VEGF, ERBB3, and TNF-α were found in the research group than those in the regular group. Higher levels of IFN-γ and IL-10 were found in the former than those in the latter. The research group got higher scores of QLQ-C30 than the regular group. Conclusion: Liposome doxorubicin + ifosfamide can improve the clinical efficacy on patients with OS and improve their recovery and life quality.

Keywords: Ifosfamide, liposome doxorubicin, inflammation, osteosarcoma

Introduction

As a primary malignant bone tumor originated from mesenchymal tissue, osteosarcoma (OS) is the most common tumor among children and adolescents [1]. OS is relatively rare, but still has over 20,000 new cases each year in China [2]. Surgery is the primary treatment for OS, but the postoperative recurrence and metastasis are still high. The 5-year survival rate, lower than 30%, is far from satisfactory [3, 4]. For metastatic or locally unresectable cases, chemotherapy is the major treatment option, but there lacks a standard chemotherapy scheme [5].

Many first-line chemotherapy drugs are available for OS, including cisplatin, methotrexate, ifosfamide, and adriamycin [6]. Most clinical chemotherapy schemes are developed based on methotrexate. Long-term use of methotrexate in large doses exerts serious toxic and side effects. These can trigger severe liver and kidney injury, oral mucositis, bone marrow suppression, and digestive tract injury, compromising the life quality of patients [7-9]. Ifosfamide is a cell-cycle-phase nonspecific anti-tumor drug. As an alkylating agent, it can be hydrolyzed by phosphoramidase in the human body into a phosphoramide nitrogen mustard and play an anti-tumor role [10]. Doxorubicin, also known as adriamycin, can fight tumors by inhibiting the synthesis of DNA, RNA, and protein in tumor cells, but it exerts strong toxic and side effects [11]. Doxorubicin and ifosfamide have been used for the treatment of osteosarcoma

for decades. There is controversy over whether doxorubicin or a combination of doxorubicin and ifosfamide should be routinely used. Previous studies have shown that ifosafamide combined with doxorubicin can provide a better response rate and median progression-free survival for patients with soft tissue sarcoma than doxorubicin alone, but it can also bring more toxic side effects [12]. Liposome doxorubicin is a new type of doxorubicin drug, which retains the broad-spectrum anti-tumor effect of ordinary doxorubicin while having a lighter drug toxicity [13]. We believe that the treatment of osteosarcoma patients with liposome doxorubicin combined with ifoshamide can improve the therapeutic effect without causing unacceptable toxic and side effects. The mechanism for the development and progression of OC has not been fully clarified. The imbalance of inflammatory regulation is considered one of the main factors [14].

In this retrospective study, we analyzed the clinical data of 86 patients with OS treated by ifosfamide combined with liposome doxorubicin to evaluate its short-term efficacy, safety, and the changes of serum inflammatory cytokines.

Materials and methods

Research subjects

In this retrospective study, the clinical data of 86 patients with OS who received chemotherapy in the Honghui Hospital, Xi'an Jiaotong University from Jan. 2017 to Dec. 2019 were analyzed. Patients treated by conventional doxorubicin + ifosfamide were assigned to the regular group (n=40). Other patients treated by liposome doxorubicin + ifosfamide were assigned to the research group (n=46) based on treatment methods. The inclusion criteria: Patients confirmed with OS by medical imaging and histopathological examination; patients who met the requirements of chemotherapy; patients whose estimated survival time was ≥6 months; and patients with Eastern Cooperative Oncology Group (ECOG) score ≤1, and Karnofsky Performance Scale (KPS) score ≥70 points. The exclusion criteria: Patients with other comorbid bone tumors, bone metastasis of other malignant tumors or bone tuberculosis; patients complicated with severe osteoporosis; patients with contraindications to drugs adopted in this study; patients with serious diseases in important organs of the body; patients with loss of consciousness, mental disease or communication obstacle; patients who had not received any anti-tumor treatment within 4 weeks; patients who voluntarily gave up treatment or could not be contacted halfway; and pregnant or lactating women. This study was conducted with approval from the Ethics Committee of Honghui Hospital, Xi'an Jiaotong University. Informed consent forms were obtained from all patients and their families after they were apprised of the study.

Treatment

Patients in the regular group were administered doxorubicin + ifosfamide. Specifically, ifosfamide (Jiangsu Heng Rui Pharmaceutical Co., Ltd., State Food and Drug Administration (SFDA) approval number: H10950292) was intravenously injected into each patient at 1.5 g/m^2. One course consisted of 5 days of continuous treatment and 3 consecutive weeks without medication. Doxorubicin hydrochloride (Zhejiang Hisun Pharmaceutical Co., Ltd., SFDA approval number: H33021979) was intravenously injected into the patient at 60-75 mg/m^2. Another course consisted of 3 days of continuous treatment and 3 consecutive weeks without medication. Each patient was treated for 6 continuous courses.

Patients in the research group were administered liposome doxorubicin combined with ifosfamide. The usage and dosage of ifosfamide were the same as those of the regular group. Liposome doxorubicin injection (Shanghai Fudan Zhangjiang Biomedical Co., Ltd., SFDA approval number: H20084432) was intravenously administered at 50 mg/m^2. One course consisted of 3 days of continuous treatment and 3 consecutive weeks without medication. Each patient was also treated for 6 courses.

Outcome measures

ECOG performance status and KPS score: The KPS score [15] was used to evaluate the functional status of patients before and after treatment. The total score is 0-100. The higher the score was, the better the functional status. The ECOG score [16] was used to evaluate the physical condition of patients before and after treatment. The total score is 0-5, and the score is in

reverse proportion to the physical condition of the patient.

Clinical efficacy: After therapy, the clinical efficacy was evaluated based on the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) [17]. This was classified into complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). The total effective rate = the number of patients with CR and that of patients with PR/the total number of patients ×100%.

Adverse reactions: Adverse reactions, including myelosuppression, liver and kidney function injury dermatitis, diarrhea, vomiting, dry mouth, sore throat, leucopenia, and thrombocytopenia, of the two groups during therapy were recorded.

Quality of life: The EORTC Quality of Life Questionnaire (QLQ-C30) [18] was used to evaluate the quality of life of patients, covering body, role, emotion, cognition, and social activity. A higher score indicated a better quality of life.

Tumor markers and inflammatory factors: Corresponding ELISA kits (Wuhan Elisalab biotechnology Co., Ltd.) were adopted to determine the levels of serum tumor specific growth factor (TSGF; Cat. No. SP10390), vascular endothelial growth factor (VEGF; Cat. No. JYM-0103Hu), erb-b2 receptor tyrosine kinase 3 (ERBB3; Cat. No. JP30246), tumor necrosis factor-α (TNF-α; Cat. No. JYM0110Hu), interferon-gamma-γ (IFN-γ; Cat. No. JYM0162Hu), and interleukin-10 (IL-10; Cat. No. JYM0155Hu). The TSGF ELISA kit was purchased from Wuhan Saipei Biotechnology Co., Ltd., China. The other ELISA kits were all from Wuhan ColorfulGene Biological Technology Co., Ltd., China. Before therapy and on the first day after therapy, 3 ml fasting peripheral venous blood was sampled from each patient in the two groups in the morning, followed by 15 min centrifugation (1,000×g, 4°C) for supernatant collection. Afterwards, standard and test samples were prepared under the instructions of ELISA kits. They were then transferred to corresponding plates, followed by 30 min incubation (37°C). The plates were washed with washing liquid. Then, the incubation was carried out for another 30 min after each well was added with enzyme-labeled reagent. After washing, chromogenic reagent was added into each well, followed by 15 min incubation and addition of stop solution into each well.

Follow-up: A 2-year follow-up was conducted in the two groups by outpatient service, telephone call, and visit once every month. Based on the follow-up results, a 2-year overall survival (OS) curve was established for the patients. OS was defined as the period from the starting of therapy to the death of the patient or the date of the last follow-up.

Statistical analyses

SPSS 18.0 (EASYBIO Company) was adopted for statistical analyses of data, and GraphPad Prism 7 was adopted for drawing of corresponding figures. The chi-square test was adopted for inter-group comparison of counting data [n (%)]. The inter-group comparison of measured data ($\bar{x}$±sd) was conducted using the independent-samples T test. The intra-group comparison was conducted using the paired-t test. The Kaplan-Meier method was used to draw the OS curve of patients based on the 2-year follow-up. The Log-rank test was used to analyze the difference between the two groups in survival. $P<0.05$ implied a remarkable difference.

Results

Comparison of general data

No notable difference was found between the two groups in general data including gender, age, weight, KPS score, Enneking stage, ECOG score, and tumor location (all $P>0.05$, **Table 1**).

Clinical efficacy

According to the evaluation results of clinical efficacy on the two groups, the regular group showed a total effective rate of 65.00%, with 10 cases of CR (25.00%), 16 cases of PR (40.00%), 10 cases of SD (25.00%), and 4 cases of PD (10.00%). The research group showed a total effective rate of 84.78%, with 18 cases of CR (39.15%), 21 cases of PR (45.65%), 5 cases of SD (10.87%), and 2 cases of PD (4.35%). The research group presented a higher total effective rate than the regular group ($P<0.05$, **Table 2**).

Adverse reaction

During the therapy, all patients tolerated the drugs, without drug allergy. No notable difference was found between the two groups in the incidences of bone marrow suppression, sto-

Table 1. Comparison of general data ([n (%)], $\bar{x}$±sd)

Group	Regular group (n=40)	Research group (n=46)	χ^2/t	*P*-value
Gender			0.360	0.549
Female	14 (35.00)	19 (41.30)		
Male	26 (65.00)	27 (58.70)		
Age (Y)	38.88±18.30	37.30±20.34	0.376	0.708
Average weight (kg)	71.05±9.18	72.45±8.68	0.726	0.470
KPS score	82.41±4.64	82.55±4.86	1.303	0.195
Enneking staging			0.806	0.369
IIB	26 (65.00)	34 (73.91)		
Stage III	14 (35.00)	12 (26.09)		
ECOG score			0.934	0.334
0	18 (45.00)	16 (34.78)		
1	22 (55.00)	30 (65.22)		
Tumor site			0.055	0.973
Femur	29 (72.50)	34 (73.91)		
Tibia	5 (12.50)	5 (10.87)		
Others	6 (15.00)	7 (15.22)		

Table 2. Comparison of clinical efficacy [n (%)]

Group	Regular group (n=40)	Research group (n=46)	χ^2	P
CR	10 (25.00)	18 (39.15)	1.946	0.163
PR	16 (40.00)	21 (45.65)	0.279	0.598
SD	10 (25.00)	5 (10.87)	2.967	0.085
PD	4 (10.00)	2 (4.35)	1.053	0.305
Total effective rate	26 (65.00)	39 (84.78)	4.537	0.033

Table 3. Comparison of the incidences of toxic and side reaction [n (%)]

Group	Regular group (n=40)	Research group (n=46)	χ^2	P
Myelosuppression	4 (10.00)	3 (6.52)	0.346	0.556
Liver and kidney function injury	7 (17.50)	2 (4.35)	3.950	0.047
Stomatitis	8 (20.00)	3 (6.52)	3.484	0.062
Diarrhea and vomiting	18 (45.00)	15 (32.61)	1.389	0.239
Thrombocytopenia	10 (25.00)	4 (8.70)	4.173	0.041
Cardiac toxicity	8 (20.00)	2 (4.35)	5.101	0.024

matitis, diarrhea, and vomiting during therapy (all P>0.05). The research group showed lower incidences of liver and kidney function injury, thrombocytopenia, and cardiotoxicity than the regular group (all P<0.05) (**Table 3**).

Serum tumor markers

Serum VEGF, TSGF, and ERBB3 levels were detected by ELISA. It was found that there were no significant differences in serum VEGF, TSGF, and ERBB3 levels between the two groups before therapy (all P>0.05). After therapy, serum VEGF, TSGF, and ERBB3 levels were decreased in both groups, with lower levels in the research group compared with the regular group (all P<0.05, **Figure 1**).

Life quality

The QLQ-C30 was used to evaluate the life quality of patients. The two groups showed no remarkable difference in scores of QLQ-C30 before therapy (all P>0.05), but the scores of QLQ-C30 in both groups greatly increased after therapy, with higher scores in the research group than those in the regular group (all P<0.05, **Figure 2**).

Inflammatory factors

According to quantification results of serum IL-10, TNF-α, and IFN-γ by ELISA, the two groups showed no statistical difference before therapy (all P>0.05). After therapy, the TNF-α level in both groups decreased and the levels of IFN-γ and IL-10 increased, with lower serum TNF-α level and higher levels of IFN-γ and IL-10 in the research group than those in the regular group (all P<0.05, **Figure 3**).

Two-year survival rate

The 2-year OS curve of the two groups revealed a higher 2-year OS rate in the research group than that in the regular group (P=0.041, log-rank test, **Figure 4**).

Discussion

Chemotherapy is the primary treatment for OS. Many chemotherapy drugs are available, but there lacks a standard treatment scheme [19].

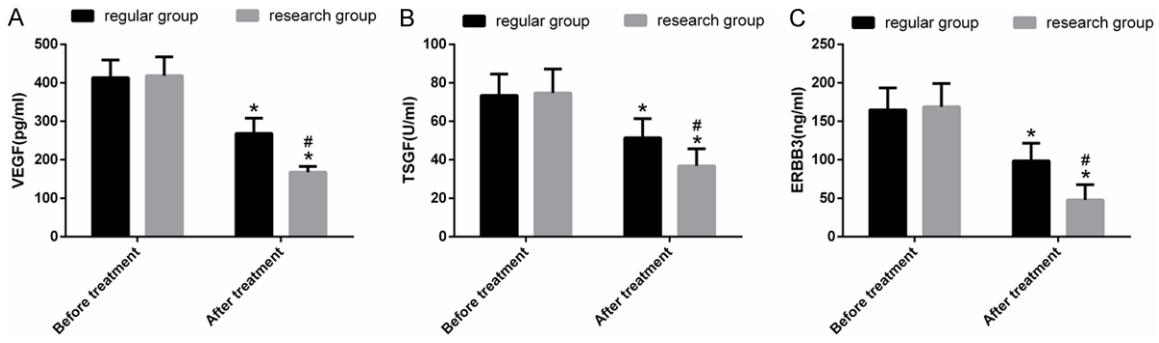

Figure 1. Serum tumor markers. A: Comparison of serum VEGF between the two groups before and after therapy. B: Comparison of serum TSGF between the two groups before and after therapy. C: Comparison of serum ERBB3 between the two groups before and after therapy. Notes: *indicates P<0.05 in intro-group comparison before and after therapy; #indicates P<0.05 *vs.* the regular group during the same period.

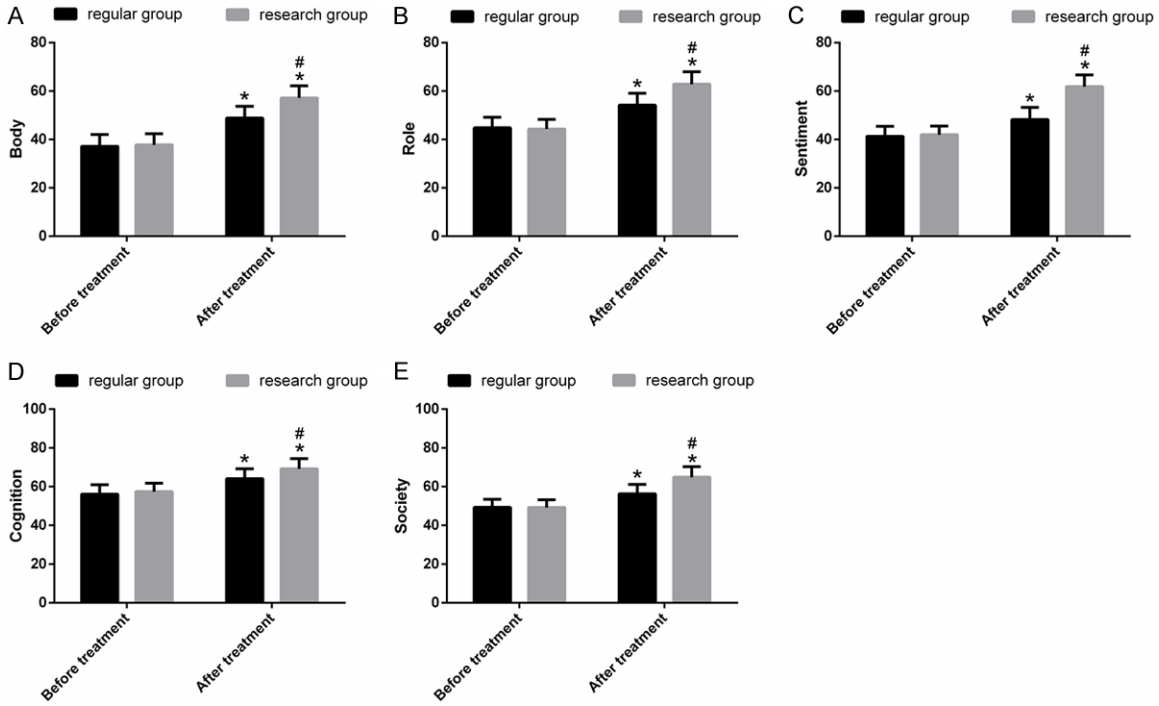

Figure 2. Comparison of life quality between two groups after therapy. A: Comparison of body scores between the two groups before and after therapy. B: Comparison of role scores between the two groups before and after therapy. C: Comparison of emotion scores between the two groups before and after therapy. D: Comparison of cognition scores between the two groups before and after therapy. E: Comparison of society scores between the two groups before and after therapy. Notes: *indicates P<0.05 in intro-group comparison before and after therapy; #indicates P<0.05 *vs.* the regular during the same period.

Liposome doxorubicin is a novel form of doxorubicin coated with liposome and modified by polyethylene glycol, with advantages of high curative effect and low toxicity [20]. Ifosfamide is an alkylating agent, without activity *in vitro,* but with an ideal anti-tumor function *in vivo*. It is usually adopted in the therapy of lung cancer, mastadenoma, and OS [21-23]. In our study, the research group showed a higher total effective rate and higher 2-year OS than the regular group. This indicated that ifosfamide combined with liposome doxorubicin was more effective than its combination with ordinary doxorubicin in the therapy of OS.

Chemotherapy can trigger side effects in different degrees, including oral mucosal disease, pain, fatigue, and dysphagia. These compro-

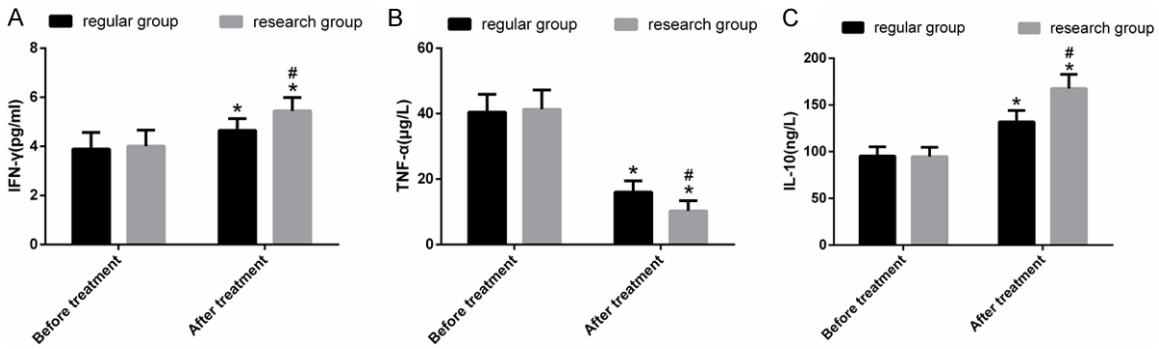

Figure 3. Inflammatory factors. A: Comparison of serum IFN-γ between the two groups before and after therapy. B: Comparison of serum TNF-α between the two groups before and after therapy. C: Comparison of serum IL-10 between the two groups before and after therapy. Notes: *indicates P<0.05 in intro-group comparison before and after therapy; #indicates P<0.05 vs. the regular group during the same period.

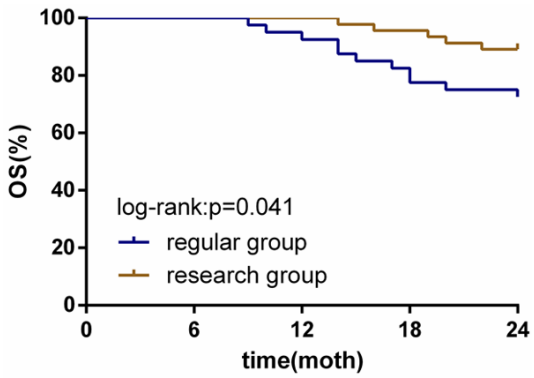

Figure 4. 2-year survival of the two groups.

mise the life quality of the patients and even threaten their life in severe cases. This study recorded adverse reactions in the two groups during therapy and found lower incidences of liver and kidney function injury, thrombocytopenia, and cardiotoxicity in the research group than those in the regular group. With the development of oncology medicine, the survival rate of OS patients has been greatly improved, making life quality important. It is necessary to guarantee the life quality of patients while prolonging their survival time. In our study, the life quality of the two groups was evaluated. The research group received higher scores in body, role, emotion, cognition, and society than the regular group after therapy.

Angiogenesis is a necessary step in tumor development, during which VEGF and its receptor (VEGFR) are irreplaceable [24]. Related studies have revealed that a higher serum VEGF level in OS patients indicated a more unfavorable prognosis [25]. TSGF is a tumor marker secreted by tumor cells during cell formation and growth, which can promote the formation, growth, and even metastasis of tumors [26]. ERBB3 is a part of the ErbB receptor family and a transmembrane tyrosine kinase receptor [27]. Prior research has revealed increased ERBB3 in various tumors including OS and the association of its high expression with the short survival rate of OS patients [28-30]. In our study, serum VEGF, TSGF, and ERBB3 in both groups declined after therapy, with lower levels in the research group compared with the regular group. The results indicated that liposome doxorubicin combined with ifosfamide can significantly inhibit the growth of OS cells and prevent the disease from exacerbation.

The imbalance of inflammation regulation is a sign of tumor development [14]. Inflammation can activate various carcinogenic processes and eventually trigger tumor progression and metastasis, such as increased tumor angiogenesis, chemotherapy resistance, and immunosuppression [31]. As a factor mainly produced by activated Th cells and NK cells, IFN-γ can stimulate the immune system to secrete many immune cytokines, resulting in the death or growth inhibition of tumor cells [32, 33]. TNF-α, mainly secreted by macrophages, is involved in inflammatory reaction and immune reaction, which shows an elevation in tumor patients [34, 35]. IL-10 is a multi-functional cytokine from multi-cell sources, which is involved in inflammatory response and immune response and recognized as an inflammatory and immu-

nosuppressive factor [36]. In our study, after therapy, a lower serum TNF-α level and higher levels of IFN-γ and IL-10 were found in the research group than those in the regular group. The results imply the positive effect of liposome doxorubicin combined with ifosfamide on inflammatory response and immune response of OS patients.

There are some deficiencies in this study. The small sample size may lead to inevitable selection bias or measured bias, which may weaken the relative reliability of the research results. Due to time constraints, patients were only followed up for 2 years. A longer-term is needed to completely understand the outcomes of the patients. Although the levels of TSGF, VEGF, ERBB, IL-10, TNF-α, and IFN-γ in serum of patients were significantly changed after treatment in this study, the specific mechanism of their changes has not been further explored. It is hoped that these limitations can be addressed by more in-depth research in the future.

To sum up, liposome doxorubicin combined with ifosfamide can contribute to higher clinical efficacy and a higher survival rate for OS patients, with fewer adverse reactions, and promote the recovery of their inflammation and immune function, to be popularized in clinical practice.

Disclosure of conflict of interest

None.

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