Int J Clin Exp Med 2020;13(2):349-357
www.ijcem.com /ISSN:1940-5901/IJCEM0104437

Review Article

Effect of gemcitabine combined with cisplatin on efficacy and serum miR-182 in patients with advanced bladder cancer

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Received November 4, 2019; Accepted December 6, 2019; Epub February 15, 2020; Published February 28, 2020

Abstract: To study the effect of gemcitabine combined with cisplatin on the efficacy and serum miR-182 in patients with advanced bladder cancer. From March 2013 to January 2016, 174 patients with advanced bladder cancer admitted to our hospital were selected as research subjects. In the control group (CG), 92 patients were treated with MVAC (vinblastine + methotrexate + adriamycin + cisplatin). In the observation group (OG), 82 patients were treated with GC neo-adjuvant chemotherapy (gemcitabine combined with cisplatin). The changes of maximum tumor diameter, clinical effect, adverse reactions, 3-year survival rate, platelet volume and coagulation indexes of patients were compared before and after treatment between the two groups. The miR-182 expression of patients in the two groups was detected by fluorescence quantitative PCR (QPCR). After chemotherapy for 6 months, the maximum tumor diameter in both groups was reduced and the reduction was more apparent in the OG ($P<0.05$). RR and DCR in the OG were higher than those in the CG after chemotherapy for 6 months ($P<0.05$). The OG was significantly better than the CG in adverse reactions of myelosuppression ($P<0.05$). After 3 years of follow-up, the survival rate of patients in the OG was higher than that in the CG ($P<0.05$). After chemotherapy, platelet volume and serum miR-182 in the two groups were lower than before chemotherapy. Fibrinogen content and coagulation indexes were higher than before chemotherapy. Platelet volume and serum miR-182 in the OG were lower than those in the CG. Fibrinogen content and coagulation indexes were higher than those in the CG ($P<0.05$). Gemcitabine combined with cisplatin is effective in the treatment of patients with advanced bladder cancer. It can regulate the expression of serum miR-182 *in vivo*. This chemotherapy regimen can be popularized in the clinic.

Keywords: Gemcitabine, combination, cisplatin, advanced bladder cancer, serum miR-182

Introduction

Bladder cancer is a common malignant tumor originating from urothelial cells in urinary system with a high incidence rate in the male population. Malignant transformation is mainly in the form of infiltration and proliferation from bladder muscularis to the abdominal cavity [1, 2]. The main clinical manifestations are intermittent and painless refractory macroscopic hematuria, which is usually relieved by itself. Some patients will not show this phenomenon, which makes diagnosis and treatment difficult. Most patients have reached advanced stages when they are diagnosed and as such have missed the best time to meet the indications for radical bladder cancer surgery, or cannot undergo surgical treatment due to being elderly and comorbid with serious diseases [3-5]. Some surgical treatments may result in severe surgical trauma, easy recurrence and many surgical complications. Bladder perfusion chemotherapy is often used to inhibit the growth of cancer cells [6, 7]. Different chemotherapy regimens will bring different toxic side effects and adverse reactions. It is of great significance to select the appropriate chemotherapy regimen to increase the survival of patients with advanced bladder cancer.

Gemcitabine is a difluoroglycoside anticancer enzyme substitute that plays an anti-tumor role by transforming the role of oxygen cytidylate kinase in the body. It mainly damages the pro-

cess of cancer cell replication, encourages tumor cell apoptosis and plays a role in controlling the progression of cancer [8]. Cisplatin is a non-specific platinum-based cell cycle anticancer broad-spectrum drug with strong sensitivity to cancer cells. Platinum atoms in cis-form bind and cross with DNA to stop replication, transcription function, mitosis and inhibit the synthesis of RNA and protein, causing tumor cells to stagnate in G1 phase [9, 10]. MiR-182 is highly conserved and abnormally expressed in various malignant tumor tissues and cells. It participates in multiple processes such as growth and differentiation of tissues and organs [11, 12]. Therefore, this experiment explored the general efficacy of gemcitabine combined with cisplatin on patients with advanced bladder cancer and analyzed the therapeutic effectiveness according to the expression changes of miR-182. The details are as follows.

Materials and methods

Datum of cases

From March 2013 to January 2016, 174 patients with advanced bladder cancer admitted to our hospital were selected as research sujects. In the CG, 92 patients were treated with MVAC. In the OG, 82 patients were treated with GC neo-adjuvant chemotherapy. There were 88 males and 86 females, with an average age of (53.51±7.72) years old. The average tumor diameter was (3.25±1.57) cm, with >3 cm in 130 cases and <3 cm in 44 cases.

Inclusion and exclusion criteria

Inclusion criteria: patients who met the criteria for advanced bladder cancer through histological or clinicopathological diagnosis were included and the stage was III-IV [13].

Exclusion criteria: (1) patients who have visited the hospitalmany times and already received anti-cancer treatment were excluded; (2) patients who did not receive treatment or who strongly request to change the treatment plan during treatment were excluded; (3) patients whose nutritional status does not meet the chemotherapy conditions before treatment were excluded; (4) patients with systemic infectious diseases were excluded. This study was approved by the Hospital Ethics Committee. All patients were informed and signed informed consent forms.

Experimental agents and instruments

Vincristine sulfate injection was purchased from Shandong Zhendong Taisheng Pharmaceutical Co., Ltd., (license number: SFDA Approval No. H14020811). Standard: 1 mg. Methotrexate injection was purchased from Ebewe Pharma Ges.m.b.H.Nfg.KG, (license number: SFDA Approval No. H20080250). Adriamycin injection was purchased from Pharmacia, (license number: SFDA Approval No. H20130-186). Cisplatin injection was purchased from Yunnan Gejiu Biological Pharmaceutical Co., Ltd. (license number: SFDA Approval No. H53021740). Gemcitabine hydrochloride injection was purchased from Jiangsu Haosen Pharmaceutical Group Co., Ltd., (license number: SFDA Approval No. H20030104). Trizol kit was purchased from Beijing Biolab Technology Co., Ltd. The cDNA synthesis kit was purchased from Agilent Technologies (China) Co., Ltd. The primers and sequencing were completed by Shanghai Shenggong Bioengineering Co., Ltd. Hematology analyzer was purchased from Beckman Kurt Company, DxH800. Related matching reagents for activated clotting time were purchased from Biolab Technology Co., Ltd.

Experimental methods

Methods of treatment: In the CG, vincristine sulfate mixture (1.4 mg/m^2 vincristine sulfate injection +30 mL 0.9% sodium chloride solution) and methotrexate mixture (1~5 g/m^2 methotrexate injection +30 mL 0.9% sodium chloride solution) were injected intravenously on the 1st, 15th and 22nd day of chemotherapy, respectively. On the 2nd day of chemotherapy, patients were given 80~120 mg/m^2 cisplatin injection +2,000 mL isotonic glucose solution and 40 mg/m^2 adriamycin injection +250 mL 5% glucose injection for instillation. In total, 22 days was a course of treatment and radical resection of bladder cancer was carried out in the second week after completing 2 courses of treatment.

In the OG, patients were given intravenous drip with 1,000 mg/m^2 gemcitabine hydrochloride and 5 mL 0.9% sodium chloride injection on the 1st and 8th day of chemotherapy, respectively.

Patients were given with 80~120 mg/m^2 cisplatin injection and 2,000 mL isotonic glucose solution on the 1st and 3rd day. A full, 22 days was a course of treatment and radical resection of bladder cancer was carried out in the second week after completing 2 courses of treatment.

Detection methods: Five mL of fasting venous blood was taken from patients before and after treatment, and then centrifuged at 30 r/min or 10 min. Serum was extracted and serum total RNA was extracted by Trizol kit. RNA concentration was measured by NanoDrop 2000 spectrophotometer and cDNA was reverse transcribed by cDNA synthesis kit. Amplification was carried out by polymerase. PCR amplification procedureswere as follows: pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, and annealing and extension at 60°C for 1 min. Micronucleus U6 RNA was used as internal reference for 40 cycles. The relative amount of miRNA was calculated by $2^{-\Delta\Delta}$CT.

Observation indexes

(1) The maximum tumor diameter from the subjects in this experiment were examined, evaluated and compared before and after chemotherapy for 6 months in this experiment. (2) The efficacy evaluation standard was evaluated according to WHO clinical guidelines for solid tumors [14]: complete remission (CR) was found when the major tumor sites disappeared and was maintained for 4 weeks. Partial remission (PR) of disease was found when the major tumor sites decreased by more than 50%. Stable disease (SD) was found when the major tumor sites increased by less than 25%. Progression of disease (PD) was found when the major tumor sites increased by more than 25% and new tumors were found. Effective rate of treatment (RR) = CR + PR. Disease control rate (DCR) = CR + PR + SD. (3) In this study, adverse reactions during chemotherapy and 3-year survival rate after chemotherapy were counted and compared. (4) Blood plasma indicator: hematology analyzer was used to detect platelet volume and fibrinogen content of all subjects before and after chemotherapy. (5) Activated partial thromboplastin time (aPTT), prothrombin time (PT) and thrombin time (TT) were mainly compared in coagulation indexes. (6) The expression levels of serum miR-182 were compared in the two groups.

Follow-up

Regular reexamination and follow-up was conducted for all subjects who received a complete course of treatment. Patients were followed up about once every 3 months in the first year after treatment according to the follow-up principle. The patient's disease was closely followed by reexamination, follow-up at home or telephone interview after chemotherapy for 6 months. In the second year, the follow-up was conducted every 6 months and the average follow-up was 3 years.

Statistical analysis

In this experiment, SPSS 19.0 statistical software was used for statistical analysis of experimental data. Chi-square test was used for counting data. Measurement data were expressed as mean number ± standard deviation. T test was used for comparison between the two groups. Independent sample T test was used between the two groups before and after treatment. K-M survival curve was used to draw the 3-year survival condition of patients with advanced bladder cancer after discharge. Graphpad prism 8 was used to draw pictures of adverse reactions, survival rate and other data. The difference was statistically significant with $P<0.05$.

Results

Comparison of baseline data

There was no significant difference between the two groups in clinical data such as age, BMI, stage, past history and differentiation degree ($P>0.05$). More details are shown in **Table 1**.

Comparison of the maximum tumor diameter of patients between the two groups before and after chemotherapy for 6 months

Before chemotherapy, the maximum tumor diameter was approximately the same in the two groups ($P>0.05$). After chemotherapy for 6 months, the maximum tumor diameter was reduced in both groups and the reduction was

Table 1. Comparison of baseline data

Grouping	CG (n = 92)	OG (n = 82)	X^2/t	P
Gender (cases)			0.020	0.886
Male	47 (51.09)	41 (50.00)		
Female	45 (48.91)	41 (50.00)		
Age/years old	53.48±7.62	53.51±7.82	0.026	0.980
BMI (kg/m^2)	19.13±2.11	19.02±2.09	0.345	0.731
Smoking history (cases)			5.754	0.994
Yes	28 (30.43)	25 (30.47)		
No	64 (69.57)	57 (69.51)		
Drinking history (cases)			<0.001	0.989
Yes	36 (39.13)	32 (39.02)		
No	56 (60.87)	50 (60.98)		
Abdominal distension and abdominal pain (cases)			0.170	0.680
Yes	41 (44.57)	34 (41.46)		
No	51 (55.43)	48 (58.54)		
Differentiation degree (cases)			0.543	0.461
Middle differentiated	21 (22.83)	15 (18.29)		
Poorly differentiated	71 (77.17)	67 (81.71)		
TNM stages (cases)			0.046	0.831
Stage III	43 (46.74)	37 (45.12)		
Stage IV	49 (53.26)	45 (54.88)		
Tumor size (cm)			0.368	0.544
>3	67 (72.83)	63 (76.83)		
<3	25 (27.17)	19 (23.17)		
Tumor location (cases)			0.142	0.931
Fundus of bladder	54 (58.70)	47 (57.32)		
Bladder body	25 (27.17)	21 (25.61)		
Trigone	13 (14.13)	14 (17.07)		
Lymph node metastasis (cases)			0.006	0.936
Yes	60 (65.22)	53 (64.63)		
No	32 (34.78)	29 (35.37)		

more apparent in the OG (P<0.05). More details are shown in **Figure 1**.

Comparison of efficacy of patients between the two groups in the first 6 months

RR and DCR in the OG were higher than those in the CG after chemotherapy at 6 months (P<0.05). More details are shown in **Table 2**.

Comparison of the overall incidence of adverse reactions between two groups of patients with advanced bladder cancer

There was no significant difference in adverse reactions such as nausea, vomiting and alopecia between the OG and the CG (P>0.05); however, the myelosuppression in the OG was significantly better than that in the CG (P<0.05). More details are shown in **Figure 2**.

Comparison of 3-year survival rate between the two groups of patients with advanced bladder cancer

Follow-up for 3 years found that the survival rate of patients in the OG (75.61%) was higher than that in the CG (52.17%), with statistical significance (P<0.05). More details are shown in **Figure 3**.

Comparison of blood plasma indicators between the two groups

There was no significant difference in platelet volume and fibrinogen content before chemo-

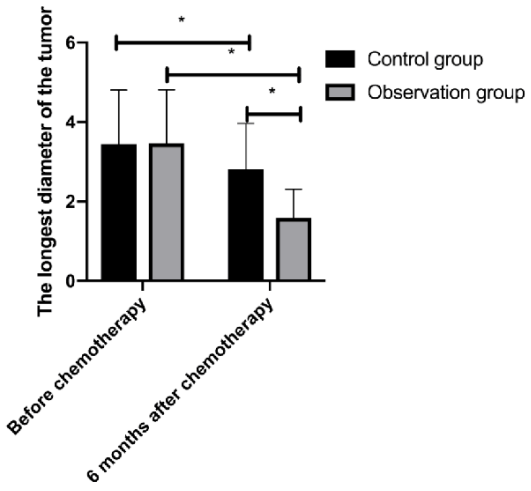

Figure 1. Comparison of the maximum tumor diameter of patients between the two groups before and after chemotherapy for 6 months. After chemotherapy for 6 months, the maximum tumor diameter in both groups reduced compared with that before chemotherapy and the maximum tumor diameter in the OG reduced significantly. Note: *represents P<0.05.

therapy between the two groups (P>0.05). Platelet volume after chemotherapy was lower than before chemotherapy in the two groups and the OG was lower. The fibrinogen content in the two groups was higher than before chemotherapy, and in the OG it was highest (P<0.05). More details are shown in **Table 3**.

Comparison of coagulation indexes between the two groups

There was no significant difference in coagulation indexes such as aPTT and PT between the two groups before chemotherapy (P>0.05). The coagulation indexes of the two groups after chemotherapy were higher than those before chemotherapy and the OG was relatively higher (P<0.05). More details are shown in **Table 4**.

Comparison of the expression levels of serum miR-182 before and after treatment between the two groups

There was no significant difference in serum miR-182 among all subjects before chemotherapy (P>0.05). After treatment, it decreased in both groups and it decreased most in the OG (P<0.05). More details are shown in **Figure 4**.

Discussion

The incidence of bladder cancer is the result of the combined action of genetic factors and external environmental factors. Bad habits such as smoking, excessive stress and drug damage are the causes of the disease [15]. Clinical diagnosis is usually confirmed by pelvic CT, B-ultrasound or cystoscopy. Other non-mechanical examinations have a lower diagnosis rate. The disease is difficult to detect. Diagnosis and treatment are difficult [16]. In the first clinical diagnosis of bladder cancer, about 25%~30% of the patients had muscular invasive cancer transformation; most patients had lymph node metastasis or even distal metastasis. The survival time of patients with locally advanced or distal metastasis was much lower than that of patients with early detection [17, 18]. Cisplatin has proven to be beneficial to the survival of patients as an adjuvant drug and a basic therapy for first-line chemotherapy. However, due to its low specificity of single therapy, it is often accompanied by the development of serious adverse reactions. So it is often combined with different drugs that coordinate the regulation of the disease for treatment [19]. Gemcitabine combined with cisplatin chemotherapy has been found to have certain control effects of disease in non-small cell lung cancer, ovarian cancer and other malignant tumors [20]. Therefore, this experiment investigated the effects of the combination of the two on serum miR-182, body function and disease change markers of patients with advanced bladder cancer to seek the detailed mechanisms of the combination therapy.

Table 2. Comparison of efficacy of treatment between the two groups in recent 6 months [n (%)]

Grouping	CG (n = 92)	OG (n = 82)	X^2	P
CR	1 (1.09)	3 (3.66)	-	-
PR	14 (15.22)	23 (28.05)	-	-
SD	27 (29.35)	36 (43.90)	-	-
PD	50 (54.35)	20 (24.39)	-	-
RR	15 (16.30)	26 (31.71)	5.711	0.017
DCR	42 (45.65)	62 (75.61)	16.181	<0.001

First of all, the changes of tumor diameter and the general efficacy of treatment in the subjects in this study showed that the maximum tumor diameter reduced in both groups after chemotherapy and the reduction in the OG was more robust. RR and DCR in the OG were higher than those in the CG after chemotherapy. Symptom control is the main goal of treatment for patients with advanced bladder cancer.

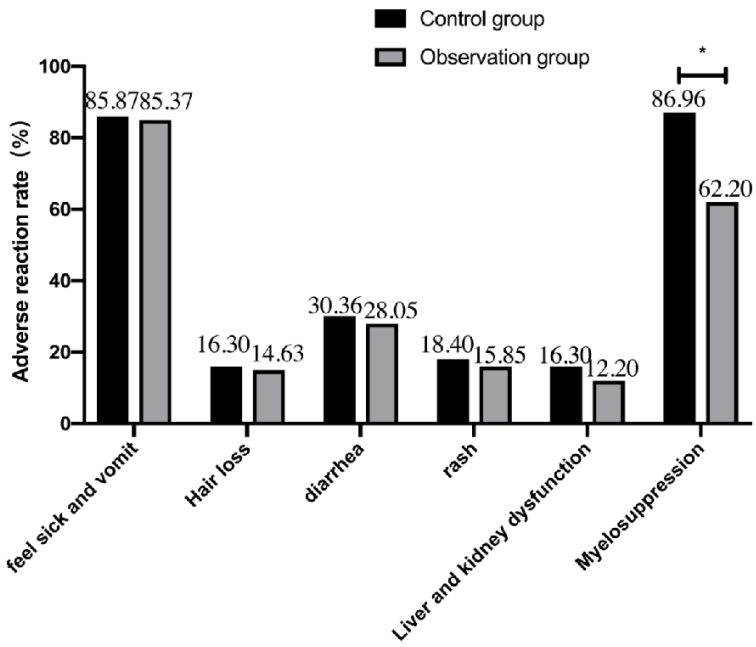

Figure 2. Comparison of the overall incidence of adverse reactions between two groups of patients with advanced bladder cancer. There was no significant difference in adverse reactions such as nausea, vomiting, alopecia, diarrhea, rash, hepatic and kidney function obstacle between the OG and the CG ($P>0.05$), but the myelosuppression in the OG was significantly greater than that in the CG. *represents $P<0.05$.

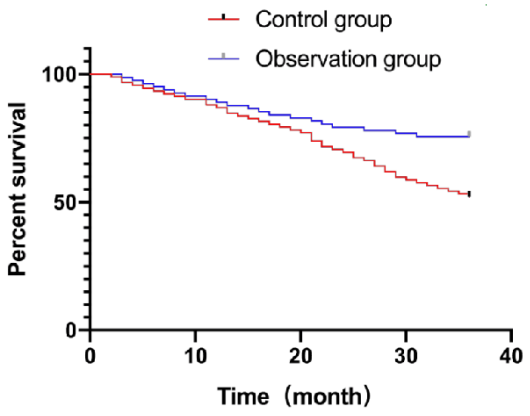

Figure 3. Comparison of 3-year overall survival of patients in both groups. Follow-up for 3 years found that the survival rate of patients in the OG (75.61%) was higher than that in the CG (52.17%).

Modern advanced chemotherapy technology can relieve the body's treatment load quantity on tumor cells, regulate clinical pathological symptoms and inhibit the proliferation of cancer cells [21]. However, there are few reports on the therapeutic and remission effects of GC and MVAC on bladder cancer. Combined with the experimental results, GC is superior to MVAC in inhibiting bladder cancer tumor and lesions metastasis. In order to further discover the toxic side effects of the two chemotherapy regimens on patients, the results showed that the OG was significantly better than the CG in myelosuppression. Some studies showed that [22], the traditional MVAC chemotherapy scheme has relatively large adverse reactions and toxic side effects on the human nervous, gastrointestinal and other systems, high risk of myelosuppression and prolonged treatment time can damage the health of patients. This showed that GC can effectively reduce the development of myelosuppression in bladder cancer treatment and the total adverse reactions are less than MVAC chemotherapy regimen. There are other similar views with these research results. Combined with results of survival rate, it was found that the survival time of patients receiving GC chemotherapy is prolonged. Upon analysis this may be related to the control of adverse reactions. In order to analyze the different connections of therapeutic effects and specific mechanisms, chemotherapy may cause changes in platelet and coagulation indexes. The results showed that the platelet volume in the two groups reduced after chemotherapy, the fibrinogen content was higher than that before chemotherapy. The changes of the two indexes in the OG were better than those in the CG and the improvement degree of coagulation indexes was better with GC. Previous studies have shown that [23, 24] when the body is in a normal state, the coagulation and anticoagulation systems are in equilibrium and stable state. The deteriorated severity of tumor is proportional to coagulation abnormalities and inversely proportional to indexes such as platelet volume. The development of this phenomenon is closely related to high blood coagulation caused by malignant tumor growth, infiltration and other lesions. It shows that the chemother-

Table 3. Comparison of blood plasma indicators between the two groups

Grouping		CG (n = 92)	OG (n = 82)	t	P
Platelet volume (fl)	Before chemotherapy	9.89±1.03	9.87±1.04	0.127	0.899
	After chemotherapy	8.67±0.93*	8.19±0.84*	3.556	0.001
Fibrinogen content (g/L)	Before chemotherapy	2.18±0.26	2.23±0.29	1.199	0.232
	After chemotherapy	2.62±0.31*	2.97±0.34*	7.103	<0.001

Note: *represents the same index between the same groups, P<0.05.

Table 4. Comparison of coagulation indexes between the two groups

Grouping		CG (n = 92)	OG (n = 82)	t	P
aPTT	Before chemotherapy	28.42±3.62	28.48±3.65	0.109	0.914
	After chemotherapy	35.73±3.76*	41.26±3.98*	9.421	<0.001
PT	Before chemotherapy	12.42±1.46	12.46±1.49	0.179	0.858
	After chemotherapy	15.36±1.53*	17.93±1.57*	10.920	<0.001
TT	Before chemotherapy	14.58±2.21	14.59±2.24	0.030	0.976
	After chemotherapy	17.26±2.38*	19.83±2.52*	6.916	<0.001

Note: *represents the same index between the same groups, P<0.05.

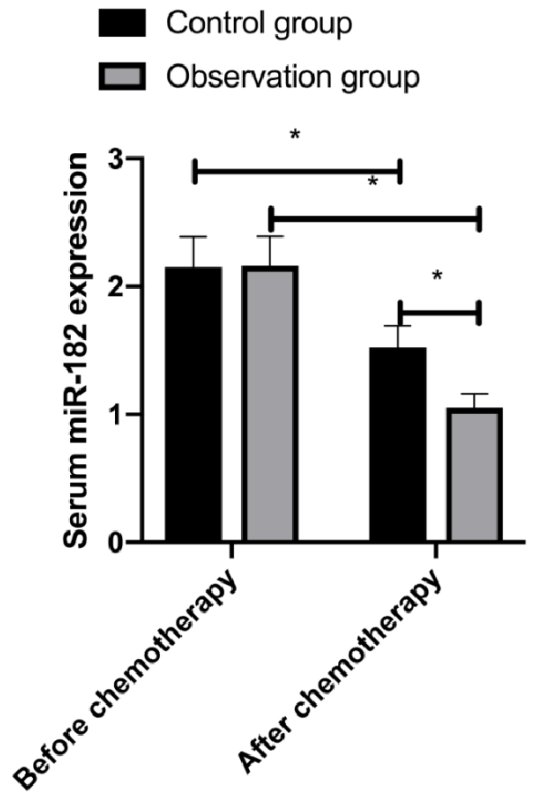

Figure 4. Comparison of the expression levels of serum miR-182 before and after treatment between the two groups. After treatment, the serum miR-182 decreased in both groups and the serum miR-182 in the OG decreased more than that in the CG.

apy regimen in the OG has greater influence on the peripheral blood and the stress response of the body and reduces the emblization caused by tumor. Previous studies have found that [25, 26], a variety of mRNA target genes can regulate lactoferrin to play the role of immune activation and platelets aggregation. However, there is no literature on miRNA-182 regulating coagulation function. The results of this study showed that miRNA-182 expression in the OG was significantly decreased. Through observation of miRNA-182, it is confirmed that the chemotherapy scheme in the OG may improve the degree of tumor deterioration and affect coagulation stability, and that the chemotherapy scheme in the OG was more suitable for patients with advanced bladder cancer.

To sum up, gemcitabine combined with cisplatin is effective in the treatment of patients with advanced bladder cancer. It can regulate the expression of serum miR-182 *in vivo*. This chemotherapy regimen can be popularized in clinic. However, there are still unresolved operational problems in this experiment. For example, the correlation analysis between miR-182 and experimental plasma, coagulation indexes has not been carried out to improve the accuracy of the correlation between miRNA and the two toensure the practicability of the experimental results; so as to improve patients' pain perception and provide research data for monitoring of patients with advanced bladder cancer from various aspects in clinic.

Disclosure of conflict of interest

None.

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