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Original Research Article

Cox regression analysis of the effect of ifosfamide and levamisole combination therapy on limb function and survival quality in osteosarcoma patients

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Abstract

Purpose: *To determine the effect of ifosfamide combined therapy on limb functionality and quality of life in patients with osteosarcoma, utilizing Cox regression analysis to identify significant prognostic factors.*
Methods: *This was a prospective analysis conducted on 90 osteosarcoma patients admitted to Shuguang Hospital, Shanghai, China between January 2021 and January 2023. Participants were randomly assigned to study (n = 49) and control groups (n = 41). The study group received a combination of ifosfamide (intravenously, 1.2 – 2.5 g/m2 of body surface area for 5 consecutive days per treatment cycle) and levamisole (50 mg) and administered 2 to 3 times daily for three days every 2 weeks from the 7th day after surgery, while the control group received levamisole alone. Functional outcome was assessed and compared using the Lysholm scoring scale, tumor necrosis factor-α (TNFα) and C-reactive protein (CRP) levels, which served as inflammatory markers in both groups. Cox regression analysis was used to identify survival predictors over a 180-day follow-up period.*
Results: *Lysholm scores in study group were significantly higher than in the control group ($p < 0.05$). Furthermore, TNFα and CRP were significantly lower in the study group compared to the control group ($p < 0.05$). There was a significant difference in the number of types of complications between both groups ($p < 0.05$).*
Conclusion: *The combination of ifosfamide and levamisole significantly enhances lower limb functionality, and decreases the incidence of complications in patients with osteosarcoma whose prognosis is intricately linked to several key factors, such as tumor location, disease staging, and the presence of complications. Further validation with larger sample sizes and multi-center studies is required.*

Keywords: *Ifosfamide, Osteosarcoma, Limb function, Survival quality, COX regression*

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INTRODUCTION

Osteosarcoma is a malignant bone tumor that originates from osteoblastic cells or primitive mesenchymal cells within the bone tissue. It predominantly affects children and adolescents, especially during the rapid growth period before epiphyseal closure. Prevalence of osteosarcoma shows a modest male predominance, and it predominantly affects individuals aged 11 to 20 years, with the next most affected age group being 21 to 30 years. As age advances, frequency of occurrence tends to decline [1-3]. Typically, this tumor is found at the proximal

segments of long bones, including the femur, tibia, and humerus. However, it is not exclusive to these locations and may also develop in other parts of the skeleton, such as the pelvis and skull. Symptoms include the presence of a mass, localized pain, restricted movement, and fractures. Treatment approach for osteosarcoma involves surgical resection of the tumor, often followed by adjuvant chemotherapy and radiotherapy.

The primary strategy for managing osteosarcoma involves surgical removal of the tumor. In cases where the disease is advanced, amputation of the afflicted limb might become necessary [4,5]. Additionally, chemotherapy and radiotherapy are utilized to target and destroy any residual tumor cells, aiming to minimize the chances of the cancer returning. However, studies show that the effectiveness of surgical treatment in curing osteosarcoma is relatively low, with success rates around 20 %. This significantly influences the overall prognosis [6,7]. Postoperative chemotherapy and radiotherapy are used as adjuvant therapies to extend patients' lifespans, benefiting from advancements in medical technology, widespread application of adjuvant chemotherapy techniques, and the development of new drugs.

Currently, chemotherapeutic drugs are commonly used including cisplatin, ifosfamide, and doxorubicin. However, the efficacy of monotherapy using these drugs is limited and may lead to adverse reactions. Therefore, combination therapy with multiple drugs is often adopted to enhance the efficacy of postoperative chemotherapy and radiotherapy for osteosarcoma. This study therefore investigated the efficacy of combining ifosfamide with levamisole in the treatment of osteosarcoma and its effects on quality of life.

METHODS

Participants

A total of 90 patients with osteosarcoma admitted at Shuguang Hospital Hospital, Shanghai, China from January 2021 to January 2023 were randomly assigned to study (n = 49) and control (n = 41) groups. The study group received ifosfamide in combination with levamisole while the control group received levamisole only. This study was approved by the Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (approval no. 202203LL-001) and conducted following the provisions in the Declaration of Helsinki [8].

Inclusion criteria

Patients who met the diagnostic criteria of osteosarcoma confirmed by both medical imaging techniques and histological analysis from biopsy specimens, patients deemed suitable candidates for receiving chemotherapy and radiotherapy based on overall health status and tumor characteristics.

Exclusion criteria

Individuals diagnosed with osteosarcoma undergoing malignant transformation into a more aggressive or different type of cancer, presence of significant comorbid conditions affecting the gastrointestinal system, liver, kidneys, heart, or lungs that could compromise treatment safety or efficacy, serious cardiovascular conditions or blood clotting disorders that elevate the risk of treatment-related complications, patients for whom surgical intervention is not recommended due to medical contraindications or risk factors, and presence of severe psychiatric or mental health disorders that may interfere with treatment adherence or ability to provide informed consent.

Treatments

Study group received intravenous infusion of ifosfamide (0.5 g; Hanhui Pharmaceutical Co., Ltd. National Drug approval no. H20084188) in combination with levamisole hydrochloride (100 mg; Beijing Shuangji Pharmaceutical Co. Ltd, National Drug approval no. H11021446). Ifosfamide was administered intravenously at 1.2 – 2.5 g/m^2 of body surface area for 5 consecutive days per treatment cycle. After each cycle, there was a three-week drug-free interval, followed by 6 to 8 sequential treatment cycles. Treatment with levamisole hydrochloride (50 mg) began on the 7th day after surgery. It was administered 2 to 3 times daily for three days every 2 weeks. Control group received only levamisole hydrochloride following the same procedure as the study group.

Evaluation of parameters/indices

Lysholm scoring scale

The Lysholm scoring scale consists of the following parameters, each assigned a score: limp (0 - 5 points), weight-bearing (0 - 5 points), locking (0 - 15 points), knee instability (0 - 25 points), pain (0 - 25 points), swelling (0 - 10 points), stair climbing (0 - 10 points), and squatting (0 - 5 points). Total score is 100, and a higher score indicates better lower limb function.

Inflammatory markers (TNFα and CRP)

Levels of inflammatory markers (TNFα and CRP) were measured to evaluate systemic inflammation. Blood samples (5 mL) were collected at baseline and 180 days after treatment, centrifuged, and analyzed for TNFα and CRP levels using enzyme-linked immunosorbent assay (ELISA).

COX survival analysis

A 180-day follow-up period was instituted to investigate Cox survival factors for patients with osteosarcoma to identify prognostic factors that influence survival rates and outcomes in osteosarcoma patients.

Statistical analysis

Data was analyzed using Statistical Packages for Social Sciences (SPSS version 25.0; IBM, Armonk, NY, USA). Continuous variables were presented as mean ± standard deviation (SD), and compared using independent sample *t*-tests. Categorical data were presented as frequency and percentages (%) and compared using chi-square (x^2) test. COX survival regression analysis was used to analyze survival quality. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

There was no significant difference in baseline characteristics (gender, location, and age) between both groups ($p > 0.05$).

Lysholm scores, TNFα, and CRP

There was no significant difference in Lysholm scores, TNFα, and CRP between study and control groups before treatment ($p > 0.05$). However, after treatment, Lysholm scores in study group were significantly higher, while TNFα and CRP levels were significantly lower compared to control group ($p < 0.05$; Table 2).

Table 1: Baseline characteristics of participants

Variable	Item	Study (*n*=41)	Control (*n*=49)	χ2	*P*-values
Sex	Female	21(51.22)	22(44.90)	0.358	0.55
	Male	20(48.78)	27(55.10)		
Location	Non-limb	16(39.02)	28(57.14)	2.933	0.087
	Limb	25(60.98)	21(42.86)		
Diameter	<10 cm	21(51.22)	25(51.02)	0	0.985
	≥10 cm	20(48.78)	24(48.98)		
Enneking stage	I	15(36.59)	11(22.45)	5.849	0.054
	II	10(24.39)	24(48.98)		
	III	16(39.02)	14(28.57)		
Surgical methods	Limb-salvage	20(48.78)	22(44.90)	0.135	0.713
	Limb amputation	21(51.22)	27(55.10)		
Chemoradiotherapy cycles	<6 weeks	18(43.90)	23(46.94)	0.083	0.773
	≥6 weeks	23(56.10)	26(53.06)		
Lymph node or distant metastasis	No	18(43.90)	22(44.90)	0.009	0.925
	Yes	23(56.10)	27(55.10)		
Age		20.32±6.21	18.67±5.81	1.295	0.199

Table 2: Lysholm scores, TNFα, and CRP in both groups

Group	Lysholm scores		TNFα (μg/L)		CRP (mg/L)	
	Before treatment	***After treatment***	***Before treatment***	***After treatment***	***Before treatment***	***After treatment***
Control (***n***=41)	37.15±6.44	54.95±9.62	40.95±6.37	9.36±1.94	24.45±0.89	13.03±2.71
Study (***n***=49)	36.49±7.32	75.16±9.88	40.01±6.56	6.09±1.18	24.46±0.88	7.39±0.86
T-value	0.447	-9.778	0.687	9.401	-0.033	12.77
P-value	0.656	<0.001	0.494	<0.001	0.974	<0.001

Treatment-related complications

There were no significant differences in the incidence of complications such as bone marrow suppression, fever, hair loss, and stomatitis between study and control groups ($p > 0.05$). Furthermore, there was significant difference in the variety of other complications encountered in study group compared to control group ($p < 0.05$; Table 3). Pareto chart analysis revealed that one or two specific complications predominated (Figure 1).

COX regression analysis of survival quality

Survival status showing 68 cases (75.6 %) alive, and 22 cases (22.4 %) deceased was taken as the dependent variable, with death as the event time. Treatment method, gender, age, tumor location, tumor diameter, Enneking staging, surgical approach, chemotherapy/radiotherapy cycles, presence of metastasis, Lysholm score, TNFα, CRP, and the number of types of complications were taken as independent variables and included in the COX survival regression model (Table 4). The findings revealed that the quality of survival in osteosarcoma patients was affected by several factors, including the approach to treatment, the tumor's location, being at Enneking stage III, the Lysholm score, levels of TNFα, and occurrence of three distinct types of complications (Table 5).

DISCUSSION

Osteosarcoma is one of the most common primary malignant tumors of bones among adolescents with high malignant severity [9]. Poor standard treatment for patients with recurrent or refractory osteosarcoma correlates with poor prognosis.

Table 3: Incidence of complications

Variable	Response	Control (*n*=41)	Study (*n*=49)	χ^2	*P*-value
Bone marrow suppression	No	38(92.68)	47(95.92)	0.445	0.505
	Yes	3(7.32)	2(4.08)		
Fever	No	36(87.80)	47(95.92)	2.049	0.152
	Yes	5(12.20)	2(4.08)		
Hair loss	No	37(90.24)	47(95.92)	1.155	0.282
	Yes	4(9.76)	2(4.08)		
Stomatitis	No	38(92.68)	48(97.96)	1.463	0.226
	Yes	3(7.32)	1(2.04)		
Abnormal liver function	No	35(85.37)	46(93.88)	1.797	0.18
	Yes	6(14.63)	3(6.12)		
Types of complications	No	24(58.54)	41(83.67)	7.998	0.046
	One	14(34.15)	6(12.24)		
	Two	2(4.88)	2(4.08)		
	Three	1(2.44)	0(0.00)		

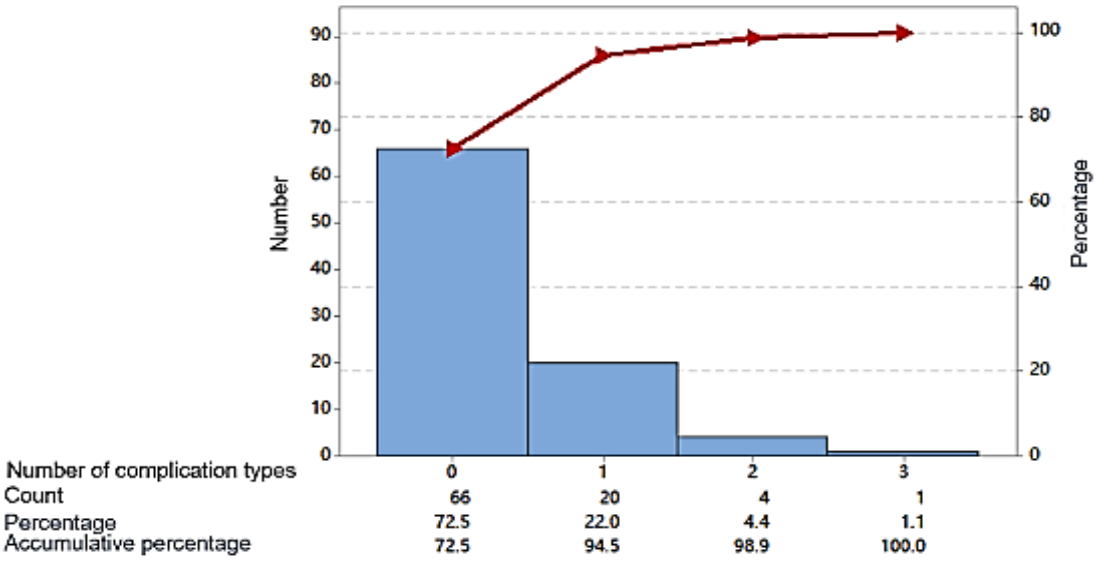

Figure 1: Pareto chart of the number of complication types

Table 4: Assignment scores of variables

Variable	Assignment scores	Reference
Survival status	Survival = 0; Death = 1	Survival
Sex	Female = 0; Male = 1	Female
Location	Non-limb = 0; Limb = 1	Non-limb
Diameter	<10 cm = 0; ≥10 cm = 1	<10 cm
Enneking stage	Ⅰ = 1; Ⅱ = 2; Ⅲ = 3	Ⅰ
Surgical methods	Limb-salvage = 0; Limb amputation = 1	Limb-salvage
Chemoradiotherapy cycles	<6 weeks = 0; ≥6 weeks = 1	<6 weeks
Lymph node or distant metastasis	No = 0; Yes = 1	No
Types of complications	No = 0; One = 1; Two = 2; Three = 3	No

Table 5: COX regression analysis of survival quality

Parameter	B	SE	Wald	*P*-value	Exp (B)	95 % CI	
						Lower	*Upper*
Group	-2.302	0.715	10.361	0.001	0.1	0.025	0.407
Sex	0.163	0.525	0.096	0.757	1.177	0.42	3.294
Age	-0.073	0.047	2.438	0.118	0.929	0.847	1.019
Location	-1.5	0.781	3.685	0.055	0.223	0.048	1.032
Diameter	0.871	0.576	2.288	0.13	2.39	0.773	7.395
Enneking Ⅱ	-0.045	0.826	0.003	0.956	0.956	0.189	4.822
Enneking Ⅲ	1.368	0.665	4.23	0.04	3.927	1.066	14.461
Surgical methods	0.475	0.623	0.581	0.446	1.608	0.474	5.449
Chemoradiotherapy cycles	-0.763	0.482	2.503	0.114	0.466	0.181	1.2
Metastasis	0.053	0.587	0.008	0.928	1.054	0.334	3.329
Lysholm scores	0.086	0.05	2.917	0.088	1.089	0.987	1.202
TNFα	0.105	0.054	3.708	0.054	1.11	0.998	1.235
CRP	-0.577	0.416	1.923	0.166	0.562	0.248	1.269
A complication	0.106	0.846	0.016	0.9	1.112	0.212	5.835
Two complications	1.534	1.449	1.121	0.29	4.636	0.271	79.286
Three complications	4.633	1.949	5.65	0.017	102.785	2.254	4686.805

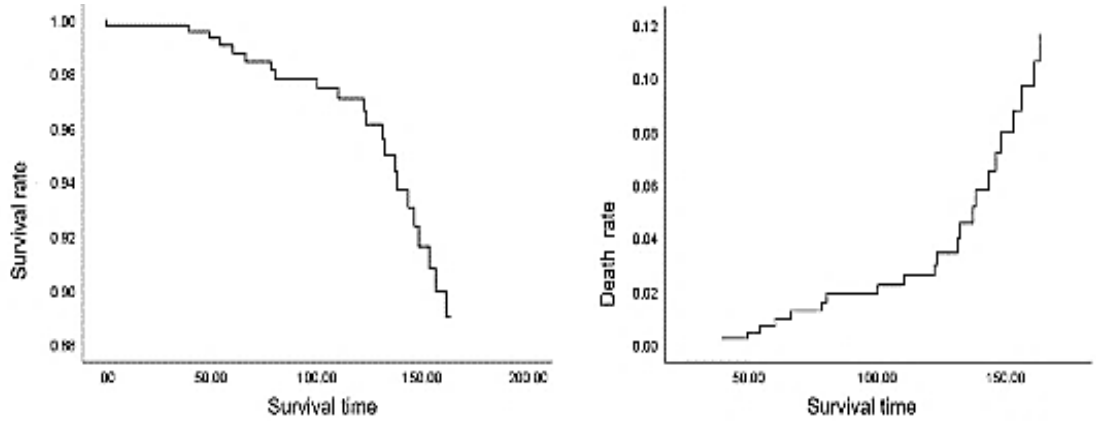

Figure 2: Survival and death Curves

As a result, new regimens are needed for patients with recurrent or refractory osteosarcoma. Ifosfamide may be used singly or in combination with other agents in sarcoma treatment [3]. The most commonly used regimen is three daily divided doses with doses ranging from 2 to 4 g/m^2/day given as an inpatient over 4 h. Over time, efforts have been made to improve treatment outcomes and prognosis. This study investigated the effect of ifosfamide combination therapy on limb functionality and quality of life in patients with osteosarcoma, utilizing Cox regression analysis to identify significant prognostic factors. The results revealed that study group (treated with a combination of ifosfamide and levamisole), exhibited significantly improved Lysholm scores after treatment compared to control group. This suggests the effectiveness of ifosfamide and levamisole in enhancing the rehabilitation of limb functionality. Ifosfamide, a potent alkylating chemotherapy agent, effectively interrupts the replication and growth of cancer cells by inducing crosslinks in DNA strands. Levamisole, in contrast, acts as an

immunomodulator, improving the immune system to effectively target and eradicate cancer cells. The synergistic combination of ifosfamide with levamisole presents significant advantages in the treatment of osteosarcoma [10-12]. Combined chemotherapy regimen of Ifosfamide and Levamisole, applied before or after surgery, improves the likelihood and efficacy of limb-saving procedures. Also, study group showed significantly lower levels of TNFα, and CRP, and fewer types of complications compared to control group. Findings indicate that the combined use of ifosfamide and levamisole in study group offers therapeutic benefits in managing osteosarcoma.

Tumor necrosis factor-α (TNFα) and C-reactive protein (CRP), as markers of inflammation, typically rise with increased inflammatory activity. Lower levels in the study group suggest that the combination therapy effectively reduces inflammatory response in osteosarcoma patients. This effect may be largely due to the immunomodulatory properties of levamisole, which reduces inflammation. However, the fewer types of complications in study group showed the superior efficacy and safety of ifosfamide and levamisole combination therapy. This improved outcome is likely a result of the synergistic effects which include halting tumor progression and enhancing immune responses, ultimately leading to lower likelihood of complications.

This comprehensive analysis utilized a COX proportional hazards regression model to investigate factors fundamental to the survival quality of individuals diagnosed with osteosarcoma. These factors encompass the method of treatment, anatomical location of the tumor, categorization as Enneking stage III, the Lysholm score, levels of TNF, and incidence of three specific complications, each contributing uniquely to the prognosis and management of osteosarcoma. The strategic selection and integration of treatment approaches are paramount in enhancing patient survival. The site of the osteosarcoma significantly influences therapeutic decisions and prognostic expectations. The Enneking Stage III indicates extensive tumor invasion and metastasis, presenting significant challenges in achieving effective local control, heightening the likelihood of lymph node and distant metastasis, and exacerbating immune suppression and inflammation.

The Lysholm score serves as a measure of joint function in evaluating the rehabilitative progress and survival quality of osteosarcoma patients. Furthermore, TNFα levels serve as an important marker for osteosarcoma. High levels of TNFα are indicative of a worsened survival quality and a less favorable prognosis. The association between elevated TNFα concentrations and negative patient outcomes underscores the importance of managing inflammation alongside direct anti-tumor strategies to improve overall survival and well-being in osteosarcoma cases [13-15].

Osteosarcoma treatment is often accompanied by several complications, including but not limited to bone marrow suppression, fever, hair loss, oral ulcers, and impaired liver function. Such complications detrimentally impact survival quality. Effective and timely intervention to prevent and manage these complications is important for enhancing patient outcomes [14]. Specifically, complications such as bone marrow suppression, fever, oral ulcers, and liver dysfunction manifest as fatigue, weakness, and a diminished appetite, thereby worsening quality of life and daily functioning. This physical decline amplifies emotional distress, further diminishing survival quality. Furthermore, hair loss and oral ulcers may lead to psychological discomfort, and affect self-image and social interaction, while oral ulcers interfere with eating and speaking, contributing to a decline in psychological health and overall survival quality [15]. Critical complications like bone marrow suppression and liver dysfunction may necessitate a pause or reduction in treatment, potentially compromising therapy effectiveness.

Limitations of this study

This study was carried out in a single center, with a small sample size, and hence the results may have some degree of bias.

CONCLUSION

The combination of ifosfamide and levamisole for the management of osteosarcoma improves lower limb function and reduces complications in patients with osteosarcoma whose prognosis is influenced by the location of the tumor, staging, and complications. Further validation with larger sample sizes and multi-center studies is required.

DECLARATIONS

Acknowledgements

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None provided.

Ethical approval

This study was approved by the Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (approval no. 202203LL-001).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Yuwei Cai and Lin Zhou contributed equally to this work.

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REFERENCES

1. *Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: Current treatment and a collaborative pathway to success. J Clin Oncol 2015; 33(27): 3029-3035.*
2. *Liao R, Cao J. Emodin induces osteosarcoma cell apoptosis by promoting ATM protein cleavage. Trop J Pharm Res 2023; 22(1): 31-36 doi: 10.4314/tjpr.v22i1.5*
3. *Fan XL, Cai GP, Zhu LL, Ding GM. Efficacy and safety of ifosfamide-based chemotherapy for osteosarcoma: a meta-analysis. Drug Des Devel Ther 2015; 9: 5925-5932.*
4. *Gaspar N, Venkatramani R, Hecker-Nolting S, Melcon SG, Locatelli F, Bautista F, Longhi A, Lervat C, Entz-Werle N, Casanova M, et al. Lenvatinib with etoposide plus ifosfamide in patients with refractory or relapsed osteosarcoma (ITCC-050): a multicenter, open-label, multicohort, phase 1/2 study. Lancet Oncol 2021; 22(9): 1312-1321.*
5. *Haghiralsadat F, Amoabediny G, Sheikhha MH, Forouzanfar T, Helder MN, Zandieh-Doulabi B. A novel approach on drug delivery: Investigation of a new nano-formulation of liposomal doxorubicin and biological evaluation of entrapped doxorubicin on various osteosarcoma cell lines. Cell J 2017; 19(Suppl 1): 55-65.*
6. *Mariani E, Meneghetti A, Tarozzi A, Cattini L, Facchini A. Interleukin-12 induces efficient lysis of natural killer-sensitive and natural killer-resistant human osteosarcoma cells: the synergistic effect of interleukin-2. Scand J Immunol 2000; 51(6): 618-625.*
7. *Li Z, Xu D, Chen X, Li S, Chan M, Wu W. LINC01133: an emerging tumor-associated long non-coding RNA in tumor and osteosarcoma. Environ Sci Pollut R 2020; 27(26): 32467-32473.*
8. *World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310(20): 2191-2194.*
9. *Bao YP, Yi Y, Peng LL, Fang J, Liu K, Li W, Luo H. Roles of microRNA-206 in osteosarcoma pathogenesis and progression. Asian Pac J Cancer Prev 2013; 14: 3751-3755.*
10. *Lee JA, Lim J, Jin HY, Park M, Park HJ, Park JW, Kim JH, Kang HG, Won YJ. Osteosarcoma in adolescents and young adults. Cells-Basel 2021; 10(10): 2684.*
11. *Jia Y, Xiong Y, Peng Z, Wang G. Risk of cardiovascular death in osteosarcoma. Jcpsp-J Coll Physici 2023; 33(3): 266-269.*
12. *Chen B, Zeng Y, Liu B, Lu G, Xiang Z, Chen J, Yu Y, Zuo Z, Lin Y, Ma J. Risk factors, prognostic factors, and nomograms for distant metastasis in patients with newly diagnosed osteosarcoma: a population-based study. Front Endocrinol 2021; 12: 672024.*
13. *Da W, Tao Z, Meng Y, Wen K, Zhou S, Yang K, Tao L. A 10-year bibliometric analysis of osteosarcoma and cure from 2010 to 2019. BMC Cancer 2021; 21(1): 115.*
14. *Martins-Neves SR, Sampaio-Ribeiro G, Gomes C. Self-renewal and pluripotency in osteosarcoma stem cells' chemoresistance: Notch, Hedgehog, and Wnt/beta-catenin interplay with embryonic markers. Int J Mol Sci 2023; 24(9): 8401.*
15. *Gao Z, Yao K, Cai P, Long N, Cao Y, He F, Liu L, Xiao C. Establishment of a survival risk prediction model for adolescent and adult osteosarcoma patients. Biomed Res Int 2022; 2022: 9559496.*