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Prognostic Significance of Clinical and Post-Neoadjuvant Chemotherapy Associated Histomorphological Parameters in Osteosarcoma: A Retrospective Study from a Tertiary Care Center

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Abstract

Introduction Osteosarcoma is the most prevalent bone cancer in adolescents. Neoadjuvant chemotherapy (NACT) followed by resection is the current modality of treatment for osteosarcoma. Histological evaluation of extent of tumor necrosis on resection is a well-established prognostic indicator in osteosarcoma correlating with survival in most cases.

Objectives The main objective of this study was to establish prognostic significance of various clinical and histological parameters post-NACT in osteosarcoma and to compare the integrated prognostic index proposed by Chui et al, with grading of response to NACT by Huvos and Rosen for osteosarcoma.

Materials and Methods This is a retrospective study done over a period of four years and includes 47 cases of osteosarcoma treated with NACT. All slides were reviewed and association of various clinical and histological parameters with overall survival was assessed with chi-squared test and Cox-regression analysis.

Results Statistical analysis revealed the prognostic significance of age at presentation, anatomic site, primary tumor size, metastatic status, and clinical stage. Histological parameters such as mitosis $\geq$10/10hpfs, $\geq$10% residual tumor were significantly associated with poor survival. Tumor necrosis $\geq$ 90% (excluding areas of hemorrhage, fibrosis and acellular osteoid) was significantly associated with increased survival. An integrated prognostic index formed by combining above parameters gives a better estimate of overall survival compared with residual disease or necrosis alone.

Conclusion Integrated prognostic index improves prognostication in patients treated for osteosarcoma.

Keywords
- osteosarcoma
- Huvos and Rosen grade
- integrated prognostic index

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Introduction

Osteosarcoma is the most prevalent bone cancer in adolescents. Current modality of treatment is neoadjuvant chemotherapy (NACT) followed by surgical ablation. Traditionally, only post-chemotherapy necrosis is considered the best prognostic parameter. In addition to tumor necrosis, chemotherapy induces variable histological alterations. These changes along with various clinical parameters are of prognostic significance and an essential component for treatment stratification. The present study aims to establish prognostic significance of these parameters and also to compare integrated prognostic index proposed by Chui et al,[1] with grading of response to NACT by Huvos and Rosen,[2] and residual disease alone.

Materials and Methods

This is a retrospective study done between January 2015 and December 2018 during which 50 cases of osteosarcoma were resected at our institute which is a tertiary hospital. All cases ($n = 47$) of high-grade conventional osteosarcoma and telangiectatic osteosarcomas with preoperative NACT were included in the study. The study was designed to assess the significance of clinical and morphological features as independent (primary outcome) prognostic factors as well as in combination (secondary outcome) in treated cases of osteosarcoma. Cases diagnosed as paraosteal osteosarcomas ($n = 3$) were treated with only resection and were excluded. Clinical, gross findings were collected, microscopic findings were reviewed, and chemotherapy response was assessed for each case.

Presurgical Treatment

All the cases were treated as per the standard osteosarcoma treatment protocol.[3–8] Cases with localized disease were treated preoperatively with two cycles of Adriamycin and cisplatin followed by resection of the lesion. This was followed by four cycles of chemotherapy post-surgery. Cases with metastatic disease at presentation received four to six cycles of the above said chemotherapeutic agents upfront followed by resection of primary if possible. Since our intent of treatment is cure, dose intensity was maintained with appropriate supportive care (cisplatin 60 mg/m^2 D1 & D2 and Adriamycin 37.5 mg/m^2 D1 & D2 every 21 days).

The following clinical and morphological data was collected.

Clinical Data

Age, sex, presence or absence of metastasis, clinical stage, tumor location.

Grossing

A full-face longitudinal section/flap of ~0.3 to 0.4 cm in thickness is taken that displays the greatest extent of tumor as well as any cortical defects. Entire longitudinal section is submitted mapping the tumor with a minimum of one block per centimeter of the tumor. Areas that represent intratumoral heterogeneity are additionally sampled and a minimum of 30 sections of resected tumor were examined microscopically to quantify the tumor response.

Morphological Data

- Size of the tumor, tumor subtype, involvement of resected margins.
- Presence or absence of lymphovascular invasion, mitosis/10hpf.
- Post-chemotherapy changes such as areas of necrosis/acellular osteoid, fibrosis/ hyalinization, and residual disease were quantified separately by estimating their percentage in the slides with tumor and then mean was calculated based on the number of slides with tumor. In every case, post-chemotherapy response grade as proposed by Huvos and Rosen[2] and the integrated prognostic index as proposed by Chui et al,[1] was assessed.

Statistical Analysis

Overall survival was calculated from date of primary surgery to date of last follow-up or date of death from any cause. Association of clinical and histological parameters with overall survival assessed with chi-squared test and Cox-regression analysis using SPSS software, version 26.0 (IBM Corp., Armonk, New York, United States).

Ethics

The study was approved by the Nizams Institute of Medical Sciences (NIMS), Institutional Ethics Committee with approval number of EC/NIMS/2623/2020 dated December 2, 2020. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional) and with the Helsinki Declaration of 1964, as reviewed in 2013. Consent waiver form was obtained from the Ethics Committee due to the retrospective nature of the study.

Results

The 47 cases included in the study affected 32 men and 15 women with M: F of 2:1. Centrally located tumors affected relatively older individuals (median: 37 years, range: 20–62 years) compared with peripherally located ones (median: 17 years, range: 7–44 years.) There was no missing data for any variable included in the study.

Most common presenting complaint was pain with swelling (40/47) (85%) followed by swelling alone (5/47) (11%) and 4% (2/47) cases had a history of pathological fracture. All the cases were primary osteosarcoma without any pre-existing bone conditions. The affected sites of these cases are depicted in ►**Fig. 1**. Among peripherally located tumors ($n = 41$), the most common location was distal femur followed by proximal tibia. Among centrally located tumors ($n = 6$), mandible was the most common site. Of these 47 cases, preoperative fine needle aspiration and/or biopsy slides were available for review in 43 cases.

Median size of the tumor at resection was 9 cm with a range of 2.9 to 21.5 cm. Central tumors were of larger size (median 12 cm) compared with peripheral tumor (median

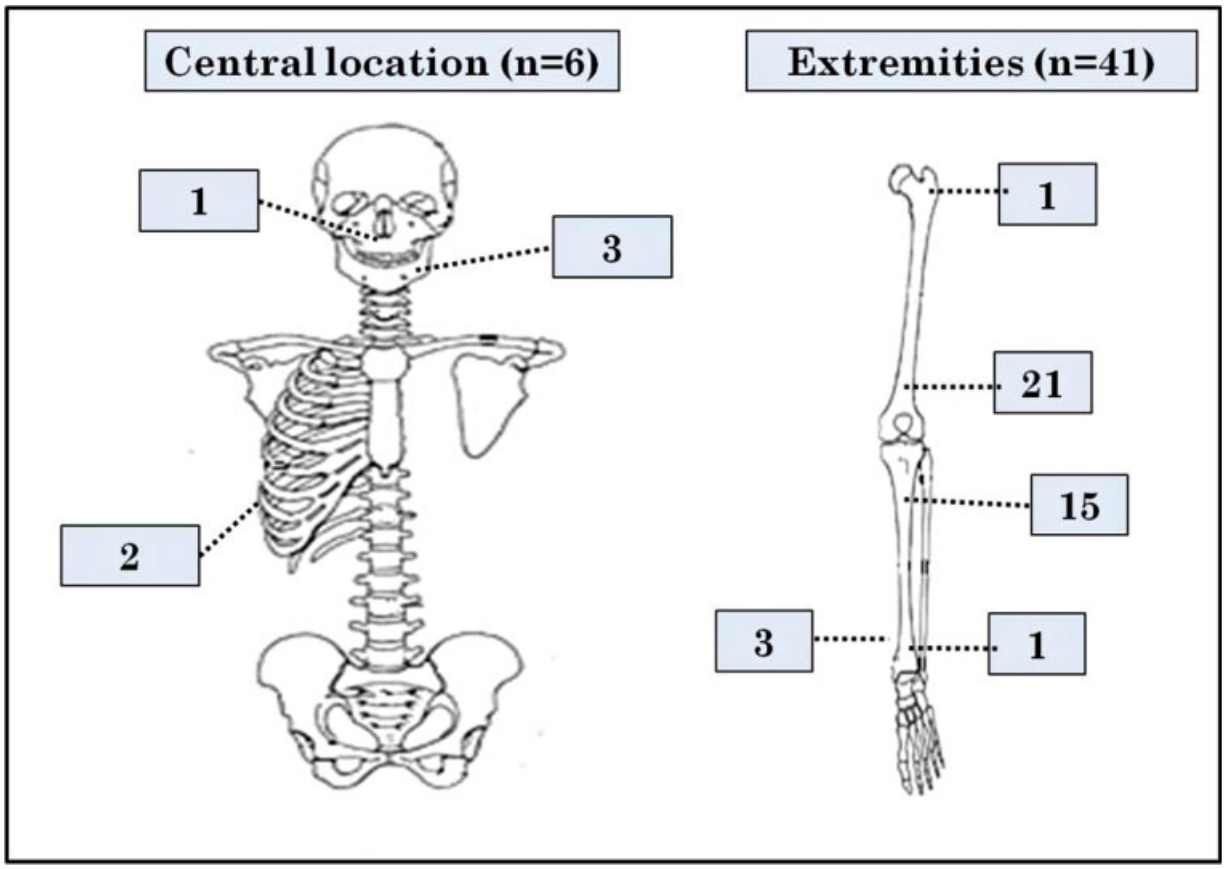

Fig. 1 Pictorial representation of tumor site distribution of osteosarcoma.

7.3 cm). The former also most often infiltrated overlying skin compared with the later (4/6 cases vs. 2/41 cases).

Histological Findings

Tumor subtyping was done on both biopsy and resection specimens. The concordance rate between initial diagnosis on biopsy and final diagnosis on resection was very high (92%). Of the 47 tumors, 42 were conventional osteosarcomas, with 35 of them having osteoblastic and seven chondroblastic morphology. The remaining five were telangiectatic osteosarcomas. Histopathological diagnosis of chondroblastic variant was made based on the predominance of chondroid matrix in the midst of neoplastic cells in ~40 to 50% of the entire specimen. Various post-chemotherapy morphological findings listed above were assessed in every case (►**Fig. 2**). Microscopic involvement of surgical resected margin was noted in 6/47 cases (12.7%).

Survival Analysis

The median duration of follow-up was 3 years with a range from 1 to 5 years. The median overall survival was 16 months with a range of 3 to 56 months. At the last follow-up, 15 patients had died, of which one had developed post-chemotherapy cytopenia with subsequent sepsis, 10 patients had metastasis, 2 had recurrences, and 2 died with the primary disease. Of the 32 patients who were alive at last follow-up, eight cases had metastasis, one had recurrence, and the remaining 23 cases were alive with no evidence of recurrence or metastatic disease. In total, 18 cases had metastasis of which 10 patients expired and the rest ($n = 8$) were alive at last follow-up. Five of the 18 cases had metastasis at presentation of which two died during the treatment and three were alive at the last follow-up with stable disease. There was no significant difference in overall survival and prognosis in patients with metastasis at presentation when compared with patients with subsequent metastasis.

Prognostic significance of each parameter is assessed in comparison with 1, 3, and 5 year's survival rates based on date of resection and with overall survival irrespective of date of resection. By chi-squared analysis, the clinical parameters (►**Table 1**), which were significantly associated with increased survival, include age at presentation <40 years ($p = 0.041$), male gender ($p = 0.05$), primary tumor size < 8 cm ($p = 0.035$), absence of metastases ($p = 0.003$), clinical stages I and II ($p = 0.002$), and tumor located in extremities ($p = 0.037$).

With respect to histological subtypes, all five cases with telangiectatic subtypes were alive at last follow-up. These patients had a favorable prognosis with better overall survival rate (100% vs. 64.3, $p = 0.043$) in comparison with conventional osteosarcomas. Histological parameters (►**Table 2**) such as mitosis ≥10/10hpfs ($p = 0.032$), ≥10% residual tumor ($p < 0.001$) were significantly associated with poor survival. Tumor necrosis ≥ 90% ($p = 0.012$) was significantly associated with increased survival. On comparison, integrated prognostic index was found to be significantly

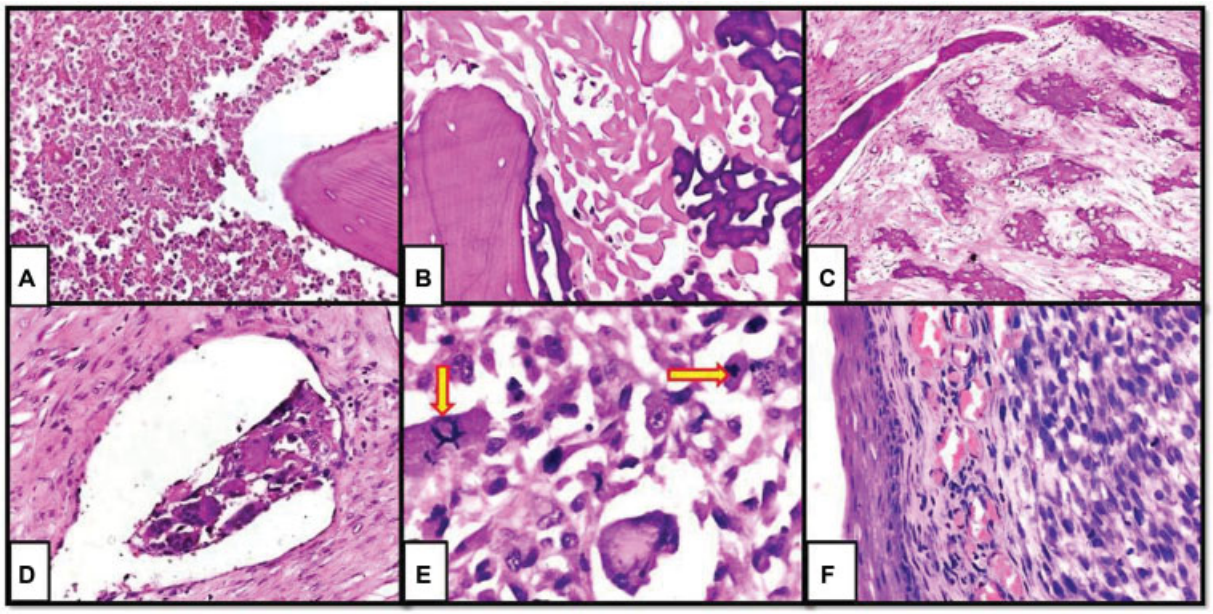

Fig. 2 Post-chemotherapy changes in osteosarcoma. (**A**) Necrosis, (**B**) acellular osteoid, (**C**) fibrosis, (**D**) lymphovascular invasion, (**E**) atypical mitosis (arrows), and (**F**) residual disease with skin involvement (x 400, hematoxylin and eosin).

better parameter than Huvos and Rosen grades in assessing survival outcome with a p-value < 0.001.

Cox-regression analysis also revealed integrated prognostic index as a significantly better prognostic parameter ($p = 0.0360$) in comparison to Huvos and Rosen grades ($p = 0.244$) and residual disease ($p = 0.099$) alone as depicted in ►**Table 3**. Hazard ratios for Huvos and Rosen grade I and II as well as the moderate and high-risk categories based on prognostic index were high indicating the poor survival outcome.

Discussion

Osteosarcoma is a primary malignant bone tumor with bimodal age distribution most commonly seen in second and third decades.[3] It has a predilection for sites with rapid growth and most proliferative growth plates such as in long bones of extremities, especially distal femur (30%), proximal tibia (15%), and proximal humerus (15%).[4] Osteosarcoma is thought to arise from primitive mesenchymal bone-forming cells, and production of malignant osteoid is its histological

Table 1 Statistical analysis by chi-squared test to assess the prognostic significance of various clinical parameters in osteosarcoma

Chi-squared analysis		1 years survival ($n = 47$)		3 years survival ($n = 20$)		5 years survival ($n = 13$)		Overall survival	
Parameter		**%**	***p*-Value[a]**	**%**	***p*-Value**	**%**	***p*-Value**	**%**	***p*-Value**
Age (in years)	<40	83	0.363	81	0.028	80	0.125	74	0.041
	>40	100		25		33		25	
Sex	Women	77	0.297	56	0.202	60	0.569	53	0.05
	Men	88		82		75		79	
Size (cm)	≤ 8 (T1)	89	0.115	100	0.001	86	0.164	82	0.035
	> 8 (T2)	77		33		50		55	
Metastasis	Absent	91	0.088	73	0.573	90	0.003	84	0.003
	Present	72		60		0		44	
Clinical stage	IA	100	0.094	100	0.015	100	0.014	100	0.002
	IIA	100		100		100		100	
	IIB	75		20		50		58	
	III/IV	72		60		0		44	
Tumor location	Extremities	86.4	0.002	81	0.028	73	0.522	75	0.037
	Head and trunk	33.3		25		50		33	

[a]*p*-Value of significance.

Table 2 Statistical analysis by chi-squared test to assess the prognostic significance of various morphological parameters in osteosarcoma

Chi-squared analysis		1 year survival ($n=47$)		3 years survival ($n=20$)		5 years survival ($n=13$)		Overall survival	
Parameter		%	*p*-Value[a]	%	*p*-Value	%	*p*-Value	%	*p*-Value
Mitosis /10high power fields	0–9	92	0.02	77	0.264	86	0.188	81	0.032
	10–19	58		67		60		42	
	≥20	100		0		0		50	
Necrosis	<50%	73	0.012	58	0.163	57	0.308	57	0.012
	≥50%	100		88		83		90	
Residual disease	< 10%	100	0.012	100	0.012	100	0.206	100	0.001
	10–49%	88		78		72		71	
	≥50%	58		20		33		33	
Huvos and Rosen score	1	71	0.205	50	0.040	50	0.359	52	0.084
	2	91		14		83		72	
	3	100		100		100		100	
	4	100		–		–		100	
Integrated prognostic index	0.1[b]	95	0.0001	100	1.002	100	0.043	90	0.0001
	2.3[c]	95		75		71		75	
	4.5[d]	40		0		0		20	

[a]*p*-Value of significance, [b](0.1), low risk, [c](2.3), intermediate risk), [d](4.5) high risk.

Table 3 Multivariate Cox-regression analysis to compare prognostic significance of Huvos and Rosen grades, residual disease with integrated prognostic index in osteosarcoma

Cox-regression analysis		1 years survival ($n=47$)			3 years survival ($n=20$)			5 years survival ($n=13$)		
Parameter		HR[a]	95% CI[b]	*p*-Value[c]	HR	(95% CI)	*p*-Value	HR	95% CI	*p*-Value
Huvos and Rosen grade	1	6.55	1.9–2.3	0.003	4.2	1.2–14.4	0.108	2.2	1.3–8.16	0.244
	2	2.45	1.25–4.68		2.86	1.23–5.25		2.12	1.35–6.14	
	3	1.65	0.96–2.78		1.15	0.92–3.1		1.53	0.94–2.18	
	4	1	–		1.12	0.85–2.3		1.09	0.83–2.5	
Residual disease (%)	<10	1	–	0.012	1	–	0.019	1.5	0.93–3.6	0.099
	≥10	5	1.4–12.4		6	1.3–16		4.2	1.2–12	
Integrated prognostic index	0.1	1	–	0.003	1	–	0.003	1	–	0.036
	2.3	3.2	1.6–5.6		2.6	1.3–5.8		2.05	1.3–4.2	
	4.5	6.55	1.9–22.3		5.2	2.2–27		4.6	1.2–18	

[a]HR, hazard ratio, [b]CI, confidence interval, [c]*p*-Value of significance.

hallmark. Most commonly, patients die of pulmonary metastatic disease. The current modality of treatment is NACT followed by surgical ablation.[5,6] Review of literature shows very few studies that explored the prognostic significance of various clinical and histological attributes post-chemotherapy.

Traditional chemotherapy response scoring system is based on tumor necrosis that was devised by Huvos and Rosen et al in 1982.[2] They assessed the histological effect of preoperative chemotherapy on the resected primary tumor and devised a grading system to assess the extent of tumor destruction. The grades devised were as follows: little or no evidence of necrosis (grade I), 50–90% necrosis among histologically viable tumor areas (grade II), predominant necrosis of 90–99% with scattered sites of viable tumor (grade III), and no histological evidence of viable tumor with 100% necrosis (grade IV). The chemotherapy effect was determined after examining a minimum of 30 representative sections from the resected specimen. Their study concluded that grade III and IV response was significantly

associated with increased disease-free survival and all cases of relapses showed a response of grade I or II.[2]

Raymond et al in their study also emphasized the prognostic significance of post-chemotherapy tumor necrosis along with other clinical and morphologic attributes such as age, sex, tumor size, tumor site, and subtype.[5] Similar results were seen in studies by Bacci et al, Harting et al, and Pakos et al.[9–11] Chui et al's study also highlighted the prognostic significance of clinical parameters such as tumor size, metastatic status, clinical stage, and tumor site.[1] Similar to these studies, the present study also revealed that age at presentation <40 years, male gender, tumor located in extremities, primary tumor size <8cm, absence of metastasis at presentation, and clinical stage I and II were significantly associated with increased overall survival. There was no significant difference in the overall survival for the two cases that had a history of pathological fracture ($p = 0.46$).

Picci et al's study particularly stressed on local recurrence in osteosarcoma in relation to histopathologic findings such as involvement of resected margins and development of a pseudocapsule around tumor.[12] A mature pseudocapsule around the tumor was not seen in cases who have not received chemotherapy or who had a poor response to it. In contrast, a well-formed, avascular, collagenous capsule was seen in patients with good response (> 90% necrosis) to chemotherapy and this was presumably related to growth arrest following therapy that resulted in sufficient time to develop a capsule. In the present study, six out of 47 cases (12.7% cases) showed tumor at surgical resection margins on microscopy of which three cases are deceased and two had recurrence. When compared with margin negative cases, there was no significant difference in the overall survival and prognosis ($p = 0.192$).

The present study revealed that among cases of osteosarcoma treated with chemotherapy, telangiectatic osteosarcomas had a favorable prognosis when compared with other subtypes of conventional osteosarcomas ($p = 0.043$). This was in line with findings of several others studies.[1,13–16] Bentzen et al stated that among conventional osteosarcomas, fibroblastic tumors had better prognosis compared with osteoblastic and chondroblastic subtypes.[17]

Although, most centers use tumor necrosis as the parameter for scoring chemotherapy response, it did not translate into survival benefit in many studies.[18–20] The possible explanation for this could be that, in tumors with necrosis <90%, major problem lies in distinguishing necrosis from other noncellular elements such as areas of hemorrhage, fibrosis, and acellular osteoid thereby leading to erroneous quantification of tumor necrosis and residual tumor. Acellular osteoid is one of the major secondary changes seen post-chemotherapy and was seen in 58% of our cases but was not associated with significant overall survival ($p = 0.085$). To address such issues, Chui et al devised an integrated prognostic index combining various clinical and morphologic attributes for effective stratification of patients post-chemotherapy.[1] This index enabled further stratification of cases into low, intermediate, and high-risk categories based on the final score. In our study, hazard ratios for Huvos and Rosen grade I and II as well as the moderate- and high-risk categories based on prognostic index were high indicating the poor survival outcome similar to their study. This new scoring system needs to be applied to larger datasets and further validation is needed.

As per Chui et al,[1] residual tumor is more reliable parameter over tumor necrosis for effective stratification of patients post-chemotherapy. When parameters of integrated prognostic index were analyzed separately, tumor size ($p = 0.035$), tumor location ($p = 0.037$), mitotic rate (0.032), and residual viable tumor ($p = 0.001$) retained significance as independent prognostic factors except for lymphovascular invasion ($p = 0.42$).

The present study demonstrated that post-NACT in osteosarcoma, >50% necrosis ($p = 0.012$), <10% residual disease ($p = 0.001$), and integrated prognostic index with low risk ($p < 0.0001$) were significantly associated with overall survival and of these, the integrated prognostic index had a slightly higher statistical significance. Several other studies also have emphasized on the percentage of residual or viable tumor post-chemotherapy as the significant prognostic parameter when compared with percentage of necrosis.[21–23] Mitoses ≥10/10 hpfs are associated with poor survival as reported by some others.[24,25] Some studies have highlighted post-chemotherapy changes such as coagulative necrosis, fibrosis/hyalinization, vascular enrichment, edematous stroma, and bizarre giant cells with degenerative features.[26–28] We encountered bizarre tumor giant cells in 44% cases.

Limitations of the Study

1. The study is limited in terms of the sample size; however, the number of cases studied were comparatively higher than other similar studies.
2. This study has short follow-up. The future research direction is to extend the study with longer follow-up, for an additional 10 to 15 years.

Conclusion

Studies exploring the prognostic significance of various morphologic features in post-chemotherapy osteosarcoma are very few. Systematic statistical analysis in the present study revealed that age at presentation, anatomic site, primary tumor size, metastatic status, and clinical stage are parameters of prognostic significance. Necrosis (>50%), residual disease (<10%), and integrated prognostic index with low risk were significantly associated with overall survival and of these, the integrated prognostic index had a slightly higher statistical significance.

Authors' Contribution
Navatha Vangala and Shantveer G. Uppin were involved in concepts, design, definition of intellectual content, literature search, data acquisition, data analysis, statistical analysis, manuscript preparation, editing, and review. K. Nageshwara Rao, P. Chandrasekhar, and Sadashivudu Gundeti were involved in concepts, definition of

intellectual content, literature search, data acquisition, data analysis, manuscript preparation, editing, and review. Navatha Vangala and Shantveer G. Uppin have provided guarantee to this manuscript.

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Conflicts of Interest
The authors declare no conflicts of interest.

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