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ORIGINAL ARTICLE

PROGNOSTIC SIGNIFICANCE OF PROLIFERATIVE ACTIVITY (KI67 EXPRESSION) IN OSTEOSARCOMA IN CHILDREN

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ABSTRACT: BACKGROUND: Osteosarcoma is the commonest primary malignant bone tumor in children. The treatment involves extensive and sometimes life threatening chemotherapy and mutilating surgery. The study of cell proliferation by Ki67 immunohistochemistry has been extensively analyzed in different cancers as a prognostic marker but little information is available in the field of osteosarcoma. The purpose of this study was to evaluate the expression of Ki67 activity in different histological types and grades of osteosarcoma and to evaluate whether higher Ki-67 expression in osteosarcoma can predict the clinical outcome. **METHODS:** The study was conducted with 30 patients of osteosarcoma. There were 19 boys and 11girls. The mean age was 12.8 yrs. We received the specimens of incisional biopsy of the suspected lesions. All the patients were treated similarly following diagnosis (chemotherapy, definitive surgery followed by chemotherapy) and they were followed up for 24 months from the date of diagnosis. Histological type and grade were determined by examining the Hematoxylin and Eosin stained slides. Proliferative activity (Ki67 expression) was measured immunohistochemically using MIB-1 antibody. Proliferation was quantitated by counting the number of immunopositive nuclei, detected manually under light microscope. Proliferation index was evaluated in relation to histological grade and survival. **RESULTS AND CONCLUSION:** We found 12 cases of low grade and 18 cases of high grade osteosarcomas. The mean of Ki67 LI in low grade osteosarcoma was seen 9.9 whereas in high grade osteosarcoma the mean was 21.6. A significant positive correlation between Ki67 LI and histological grade was observed in osteosarcomas ($p < 0.001$). There was no statistically significant correlation found between Ki67LI and the histologic types of osteosarcomas. We followed up all the cases for 24 months; a worse prognosis was observed in osteosarcomas of higher proliferative activity.
KEYWORDS: Osteosarcoma, Ki67 expression, prognostic significance, proliferative activity

INTRODUCTION: Osteosarcoma is the commonest primary malignant bone tumor.[1,2] It tends to affect mainly young children and adolescents.[3,4] The prognosis is generally poor.[5,6] The estimation of biological behavior and prognosis in osteosarcomas is a great challenge to both the pathologist and the surgeon.

Many researchers have proved that the biological behavior of a tumor is mainly determined by its rate of cell proliferation.[7, 8] The value of cell proliferation markers and Ki-67 immunostaining has been found to be prognostic in many different tumor types such as carcinoma, lymphoma, sarcoma and the brain tumors.[9-12] But in the field of osteosarcoma, only few data have been reported till today regarding its cell proliferation and the prognosis.[13]

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Moreover, the division of osteosarcoma according to histological grade is not well established. There is lack of morphologic criteria of indicating different grades of this neoplasm.[14] Therefore it is very important to search for parameters that can predict the clinical course in osteosarcoma. This study was done to evaluate the Ki67 expression in different histological types and grades of pediatric osteosarcomas and its correlation with the survival.

MATERIALS & METHODS: We received samples from 30 patients of osteosarcoma during the study period of two and half years. After clinical and radiological diagnosis, the biopsy was taken. Hematoxylin and Eosin stained slides were examined for making the diagnosis (both typing and grading) and for ensuring that the sections were representative of the tumor. The well fixed slides with non-necrotic areas were chosen for immunostaining. Sections taken on poly-L-lysine coated slides for Ki67 immunohistochemical staining.

The staining was done by standard protocol using MIB-1 antibody (Biogenex, USA). The Ki67 expression was evaluated under light microscope. All the labeled nuclei of the tumor, regardless of staining intensity, were considered positive. The percentage of positive cells was calculated, by counting 500 tumor cells. In those instances where multiple samples were analyzed from the same lesion, the highest value of Ki67 LI was considered. All the patients were treated similarly (chemotherapy, definitive surgery followed by chemotherapy) after diagnosis.

The chemotherapy consisted of Methotrexate, Vincristine, Doxorubicin, and Actinomycin. None of the cases received chemotherapy before the diagnostic biopsy. We followed up the cases for 24 months from the time of diagnosis. Patients were seen at regular intervals at Outpatients Department of Oncology and Orthopedic Surgery in the case of recurrence. Thus, any type of recurrence was added to the clinical records and in the case of death, the cause of death was established from the death certificates.

STATISTICAL ANALYSIS: Mann Whitney U test and Wilcoxone W nonparametric test were done to compare the pairs of groups of patients. The follow up period was calculated from the time of diagnosis. The survival curves of the patients were obtained by the method of Kaplan and Meier with a 24 months follow-up. The significance of the survival curves were estimated by log-rank test.

RESULTS: There were 19 boys and 11 girls with ages ranging from 05 to 16 yrs. Amongst the 30 patients, about 60% cases appeared in the age group of 10 to 15 yrs. Most of the cases were seen to affect the lower extremity mainly, femur and tibia.

In this study, we followed the Enneking grading system of malignant bone tumor for classifying the samples into low and high grades. All the 30 cases of osteosarcomas were divided into two grades on the basis of mitotic count and cell atypia. In our study, low grade tumors were determined by uniform cell type, without atypia and few mitosis (less than 10 per 10 HPF) and high grade was determined by atypical nuclei and prominent mitosis(more than 10 per 10 HPF).[14]

Figures: 1A shows the photomicrographs of a low grade osteosarcoma with little nuclear atypia and mitotic count and Figures- 1B shows the respective immunohistochemistry (Ki67 IHC) picture with low KI67 LI. Figures: 2A shows the photomicrographs of a high grade osteosarcoma with prominent nuclear atypia and high mitotic count. Figure-2B shows the respective immunohistochemistry (Ki67 IHC) with high KI67 LI.

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We had 12 cases of low grade and 18 cases of high grade tumors. The areas showing the highest mitotic count were chosen as representative of the tumor. The range of Ki67LI among low grade osteosarcoma varied from 6.2 to 14.2 with a mean of 9.9 and the median was 10.38. In high grade osteosarcoma the range was 15.2 to 25.8 with a mean of 21.6 and median of 21.2. The statistical analysis showed significant correlation ($p<0.001$) between the mitotic count and the expression of the nuclear antigen, Ki-67. [Figure-3]

On the basis of histological features, four types of osteosarcoma cases were found. Out of total 30 cases 18 cases were of osteoblastic type; chondroblastic variety was seen in 06 cases, fibroblastic type in 03 cases and the small cell variant in 03 cases. In this study, the small cell osteosarcoma had the highest Ki67 LI; however, due to small number of cases, no statistically significant correlation was established between KI67 expression and the histologic variants or type of osteosarcoma. [Figure-4]

During follow-up of these cases for 24 months, in case of low grade osteosarcomas, systemic relapse occurred in 3 cases, 2 cases were lost to follow up and 09 patients were alive after 24 months. Amongst the cases of high grade osteosarcomas, there was evidence of relapse in 12 cases, 3 patients were lost to follow-up and 8 patients remained alive after 24 months. Number of deaths due to disease was 1 case in low grade osteosarcomas, whereas in high grade osteosarcomas, it was 7. The mean of disease free survival in low grade OS was 21 months and in high grade it was 15 months. The mean of overall survival in low grade was 23 months whereas in high grade it was 20 months. [Table-1 and Table-2]

The survival curves showed a better prognosis for the low grade tumors as compared to the high grade tumors. [Figure-5] and [Figure-6]

DISCUSSION: The availability of Ki67 monoclonal antibody has opened a new era for extensive analysis of cell kinetics in human neoplasms. Ki67 antibody reveals a nuclear antigen that is expressed in proliferating but not in quiescent cells.[15-17] Due to the fact that Ki67 can be used to determine the growth fraction (the proportion of the cells committed to the cell cycle), Ki67 is considered to be an important marker in the tumor diagnostics and prognostics. In general there is a good correlation between Ki67 staining and mitotic count.[18]

In a study by Gerdes et al, the Ki-67 monoclonal antibody was found to detect a nuclear antigen that is present only in proliferating cells.[7]

As the histological grading of osteosarcoma is not well established, it is a great challenge to us, the pathologists to delineate those patients who have worse prognosis and to treat them accordingly. Here we have attempted to quantitate the proliferative activity in osteosarcomas and to determine whether proliferation index is indeed a prognostic factor.[19]

We divided the 30 cases into two grades on the basis of nuclear atypia and mitotic count according to Enneking grading system on bone tumors and a significant positive correlation between Ki67 expression and the histologic grades in osteosarcomas was observed.

Stenzel et al studied the rate of proliferation among 64 bone tumors. They found significantly higher rate of proliferation among high-grade central osteosarcomas in comparison with low-grade osteosarcomas and other benign intraosseous bone tumors. This approach proved to be very useful in the distinction between high-grade and low-grade osteosarcomas as well as bone-forming intraosseous tumors.[20]

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Our immunohistochemical results were similar to Scotlandi et al.[14] They also found a remarkable difference in Ki67 expression between low grade and high grade osteosarcomas. The Ki67 LI was found to be lower in chondroblastic osteosarcomas in comparison with osteoblastic and fibroblastic types in their study. In our study, the fibroblastic type was found to have the lowest Ki67 LI in comparison to other types. However, no significant statistical correlation could be established due to small number of cases. In our study, small cell osteosarcomas appeared to have the highest Ki67 expression, again this was not statistically significant.

We also studied the prognostic significance of Ki67 immunostaining in osteosarcoma in children. Cardoso et al[13] evaluated histological, clinical and immunohistochemical parameters of osteosarcoma, using Ki-67 labeling index (LI). They concluded that the Ki-67 LI could be useful as a prognostic marker in patients without metastasis at diagnosis. In the study by Jong et al on Ki-67 expression and outcome in high grade osteosarcoma in 27 cases, it was suggested that the tumor proliferation rate did not appear to be prognostic for high grade osteosarcomas and assessment of this feature requires further study in conjunction with other tumor characteristics.[22]

In our study, the Kaplan – Meier survival curves showed a better prognosis for the slowly proliferating osteosarcomas which had low mitotic count and low Ki67 LI as compared to highly proliferating tumors. The study has shown the correlation between grades and survivals of patients were statistically significant. Ueda et al.[21] have previously reported the prognostic significance of Ki67 expression in soft tissue sarcomas. In the study by Scotlandi et al., Kaplan-Meier survival curves showed a better prognosis for the slowly proliferating osteosarcomas. In a study by Ansari et al, the grade of the osteosarcomas was found to be a significant prognostic factor for the relapse of disease.[23]

In our study, we found 12 cases of relapse out of 18 cases among high grade osteosarcoma in comparison to only 3 cases out of12 cases amongst the low grade osteosarcomas.

A major limitation of the study was small number of cases and the study period was very short. There was significantly high rate of lost to follow up cases. The patients have a tendency not to complete their treatment as soon as they feel better and then return later with extensive disease. The treatment is also expensive. The financial burden is also a cause of incomplete follow up. Lack of knowledge regarding regular follow up, especially when the treatment is deemed completed, is another important factor of incomplete treatment.

CONCLUSION: We conclude that the Ki67LI could be conveniently used for a better definition of tumor grades, in addition to conventional histopathologic evaluation in osteosarcomas. We also think that biological aggressiveness of osteosarcomas, reflected by high Ki67 LI, is clinically relevant and bears a prognostic significance. We also feel that there is a need for increasing awareness among general public for meticulous follow up.

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Fig. 3: Box-whisker plot showing percentage (%) of Ki67 expression in relation to histopathologic grades of Osteosarcomas:

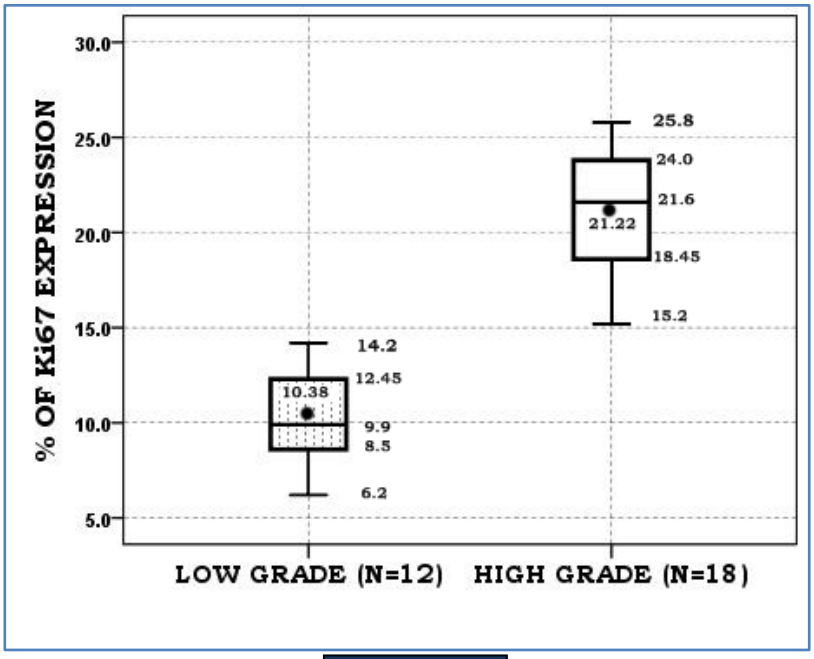

Figure 3

Fig. 4: Box-whisker plot showing percentage (%) of Ki67 expression in relation to histopathologic types of Osteosarcomas:

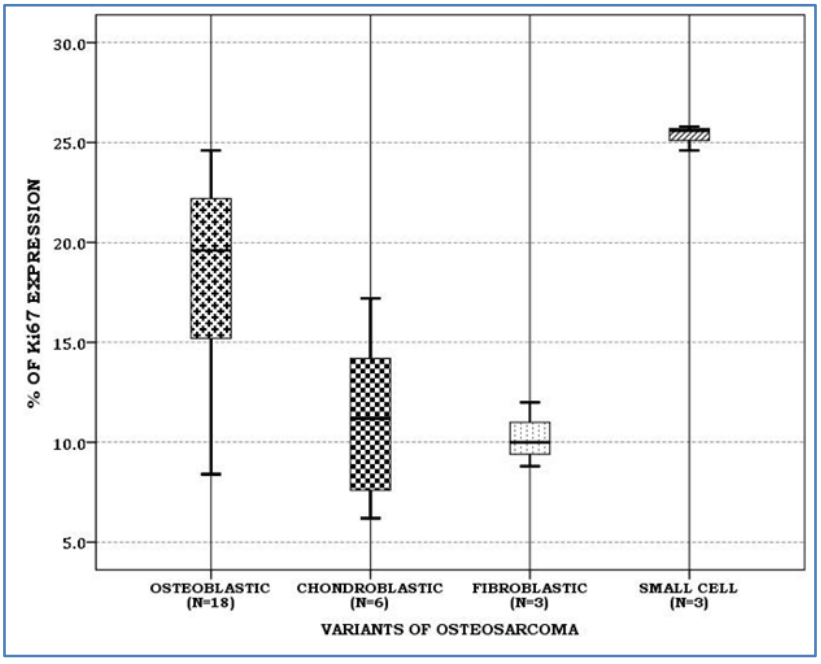

Figure 4

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Sl No.	Age (yrs.)	Sex	Ki67LI	Grade	Systemic relapse(months)	Outcome & Overall survival(months)
1	12	M	11.4	Low	No	Alive(24)
2	11	M	14	low	Relapse(12)	Death(22)
3	15	F	9.6	Low	No	Alive(24)
4	13	F	8.4	Low	No	Alive(24)
5	09	M	10	Low	Lost(21)	Lost(21)
6	14	F	12	Low	No	Alive(24)
7	18	M	6.2	low	No	Alive(24)
8	12	M	12.6	Low	Relapse(17)	Alive(24)
9	11	M	14.2	low	Relapse(14)	Alive(24)
10	15	M	9.8	Low	Lost(18)	Lost(18)
11	14	F	7.6	Low	No	Alive(24)
12	16	F	8.8	Low	No	Alive(24)

Table 1: Study of disease free survival and overall survival among low grade osteosarcomas

Sl No.	Age (yrs.)	Sex	Ki67LI	Grade	Systemic relapse(months)	Outcome & Overall survival(months)
1	17	M	18	High	Relapse(10)	Death(15)
2	13	M	17.2	High	Lost(13)	Lost(13)
3	14	M	24.6	High	Relapse(15)	Alive(24)
4	12	F	19	High	No	Alive(24)
5	10	F	21.8	High	Relapse(12)	Alive(24)
6	14	M	18.6	High	Relapse(11)	Death(15)
7	12	F	15.2	High	No	Alive(24)
8	08	M	25.6	High	Relapse(08)	Death(13)
9	15	M	18	High	Relapse(12)	Alive(24)
10	18	F	21.4	High	Relapse(17)	Alive(24)
11	12	M	22.6	High	No	Alive(24)
12	11	F	23.8	High	Relapse(13)	Death(21)
13	05	F	20.4	High	Relapse(11)	Death(15)
14	14	M	22.2	High	Relapse(09)	Death(16)
15	17	M	25.8	High	Relapse(08)	Death(13)
16	10	M	23	High	No	Alive(24)
17	13	F	24.6	High	Lost(11)	Lost(11)
18	11	M	20.2	High	Relapse(12)	Lost(15)

Table 2: Study of disease free survival and overall survival among high grade osteosarcomas

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Fig. 5**:** The Kaplan- Meier curve showing the comparison of disease free survival between low grade and high grade osteosarcomas.

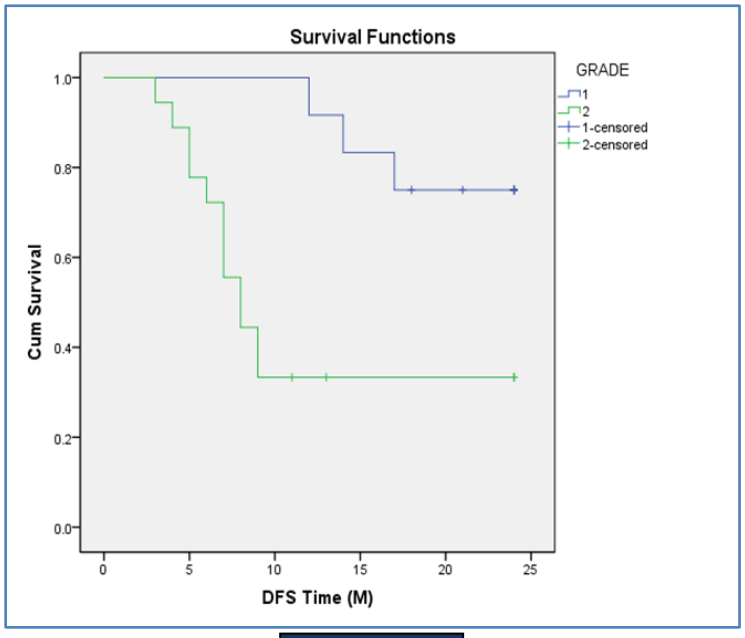

Figure 5

Fig. 6: The Kaplan- Meier curve showing the comparison of overall survival between low grade and high grade osteosarcomas.

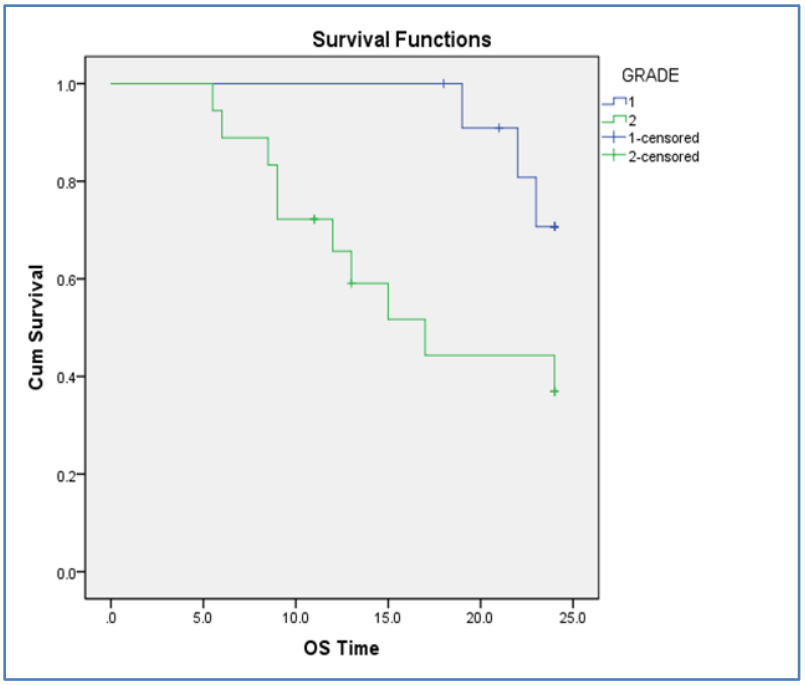

Figure 6

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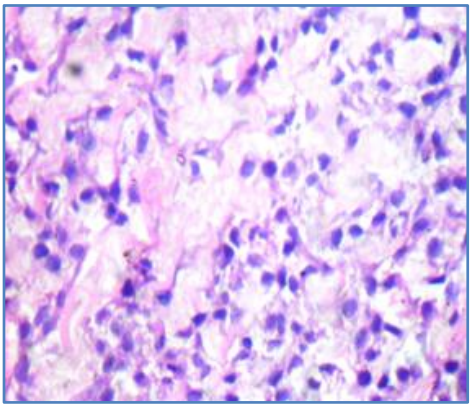

Figure 1A: The photomicrograph showing low grade osteosarcoma with hypocellular stroma and slight nuclear atypia (H&Estaining ×400)

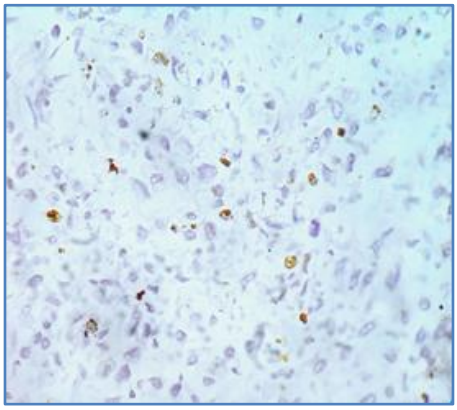

Figure 1B: The photomicrograph of the same low grade osteosarcoma showing low Ki67expression(immmunostaining×400)

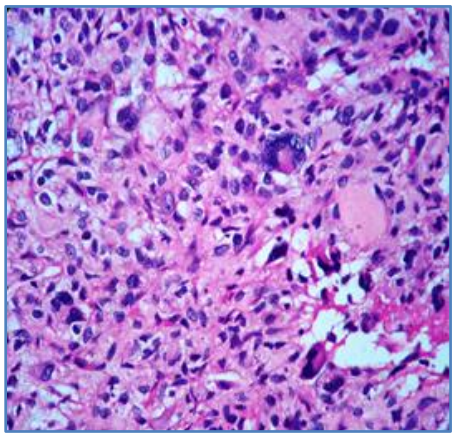

Figure 2A: The photomicrograph showing high grade osteosarcoma with prominent nuclear atypia(H&Estaining ×400)

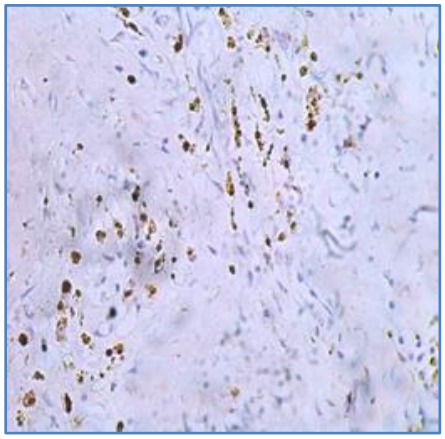

Figure 2B: The photomicrograph of this high grade osteosarcoma showing high Ki67expression(immmunostaining×400)

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