Comparative Immunohistochemical Analysis of Ki -67 in a Spectrum of Pediatric Solid Tumours

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Abstract

Introduction: The aim of this study is to analyse the clinicopathological profile of malignant pediatric solid tumours and also to evaluate the proliferative index using Ki-67 in a spectrum of these tumours.

Materials and Method: 75 cases of malignant solid tumours age group less than 17 years, reported at Sri Ramachandra Medical College from Jan 2009 to Dec 2013 are included in this study. Formalin fixed paraffin embedded tissue stained with H&E were used for routine morphology. Immunohistochemical staining was done using monoclonal anti Ki 67 antibody by Streptavidin biotin peroxidase complex technique. Labelling index or growth fraction is calculated as the percentage of positive cells for 1000 cells evaluated.

Results: Among the 75 cases 52 (68%) were males and 23(32%) were females. 22(29%) were CNS neoplasms and 53(71%) were Non CNS neoplasms which included lymphoma, Ewing's sarcoma, Osteosarcoma, Wilms tumour, Soft tissue tumours, Germ cell tumours, Neuroblastoma, Hepatoblastoma & others. The labelling index was variable according to the grade of the tumours. It varied from 2 to 40% in CNS neoplasms according to the grade and about 1 to 90% in various Non CNS neoplasms.

Conclusion: Pediatric solid tumours form a wide spectrum. Ki-67 labelling index may be a useful adjunct in predicting the biological behaviour & prognosis of pediatric solid tumours.

Keywords: Ki-67, Pediatric solid tumours, prognosis

Introduction

Pediatric solid malignant neoplasms are a significant cause of death among children. Identification of new biomarkers that can detect cancer earlier, monitor disease progression or serve as a surrogate marker for prognosis & prediction will enable to personalise medicine & improve care of the affected children. Pediatric cancers are mainly of embryonic in origin & are generally derived from nonectodermal embryonal tissues. Etiology of most childhood malignancies are unknown, some solid tumours are known to occur in association with genetic defects. For example, Beckwith Widemann, WAGR & Denys Drash syndrome are associated with Wilms tumour.

The main categories include brain tumours, neuroblastoma and ganglioneuroma, Lymphomas, Ewing's sarcoma/ PNET, Wilm's tumor, rhabdomyosarcoma, germ cell tumor, osteosarcoma, & hepatoblastoma. Pediatric brain tumours accounts for 20% of all pediatric malignancies.[1] The most prevalent primary brain tumours among the pediatric population are astrocytomas, ependymomas, medulloblastomas, glioblastoma multiforme, craniopharyngiomas, choroids plexus neoplasms, etc.

Cell kinetic data is an important indicator of the aggressiveness of tumour & clinical response. The old & widely used method for assessing cell proliferation is mitotic count in routinely processed H&E sections. Nowadays, in this IHC era we use Ki67 labelling index to assess the tumour proliferation index. Other nuclear antigens include Ki S1 & PCNA.

Materials and Method

This is a retrospective study which includes 75 cases of pediatric solid tumours, age group between 0 to 17 yrs who presented in SRMC medical center from 2009 to 2013. The records of the patients were retrived from pathology database and analyzed. Formalin fixed 3mm sections were stained by H&E and then by IHC with monoclonal anti Ki67 antibody using streptavidin biotin peroxidase complex. Lymphoid tissue was taken as positive control for Ki-67 immunostaining.

The labeling index or growth fraction is calculated by the proportion of positive cells in relation to a total of 1000 cells evaluated. Each slide was evaluated at 40x to find areas with maximum positive cells and then at 200x magnification to count the positive cells.

Stromal staining cells and tumour infiltrating lymphocytes are avoided from evaluation. The mean, standard deviation and standard error of mean for Ki-67 was calculated in all the pediatric solid tumors. The correlation between various grades of CNS neoplasms with Ki-67 was done using Kruskal Wallis one way analysis and Pearson correlation test.

Results

The study comprised of 75 cases of Pediatric solid tumours reported in Sri Ramachandra Medical College from January 2009 through December 2013. There were 53 males (68%) and 22 females (32%). There was a male preponderance with male to female ratio 2.3: 1. Twenty cases were less than 5 yrs, fifteen cases were

between 5 to 10 yrs and in forty cases, age ranged between 11 to 18yrs.

CNS tumours constituted 31% and non CNS tumours were 69% (Table 1). These included Lymphomas (16%), Ewing's sarcoma/PNET (12%), Osteosarcoma (8%), Wilm's tumour (6%), Germ cell tumour (5%), Soft tissue tumours (6%), and others (16%) (Table 2). The tumours belonging to others category were three cases of neuroblastoma, two cases of papillary carcinoma thyroid, one case of hepatoblastoma, malignant melanoma, squamous cell carcinoma, neuroendocrine tumour, adenoid cystic carcinoma and renal cell carcinoma. The clinical characteristics of all categories of pediatric solid tumours are mentioned in Table 5.

CNS neoplasms reported were Pilocytic astrocytoma, WHO grade 1 (3 cases), Grade II Glioma (10 cases), one case of Grade III astrocytoma and anaplastic oligodendroglioma, Medulloblastoma (5 cases) and Glioblastoma multiforme (4 cases). Among the Lymphomas, Non-Hodgkin's lymphoma (8 cases) were predominant in comparision to Hodgkin's lymphoma (4 cases). Soft tissue tumours included Botryoid RMS, Embryonal RMS, Hemangioendothelioma, Fibrosarcoma and Malignant peripheral nerve sheath tumour.

The mean Ki-67 LI for grade I, II, III & IV gliomas were 0.5%, 2.3%, 18.4% & 28% respectively (Fig. 1a, b, c). The mean proliferative index in pediatric brain tumours are depicted in (Table 3). Medulloblastomas and glioblastoma multiforme have a high proliferative index [Mean Ki-67 was 28%]. The mean Ki-67 labelling index of other pediatric solid tumours are expressed in Table 4 (Fig. 2, 3, 4). Pediatric lymphomas especially Non-Hodgkin's lymphomas and blastomas have a high proliferative index [Mean Ki-67 more than 30%].

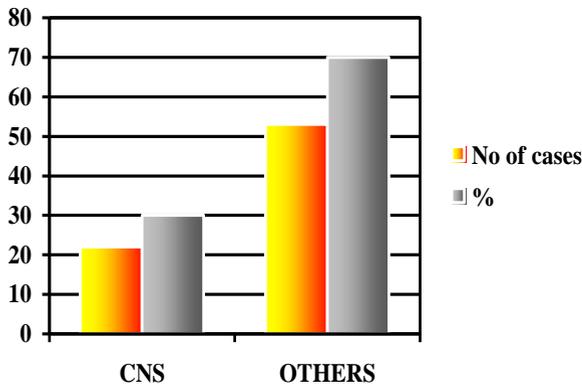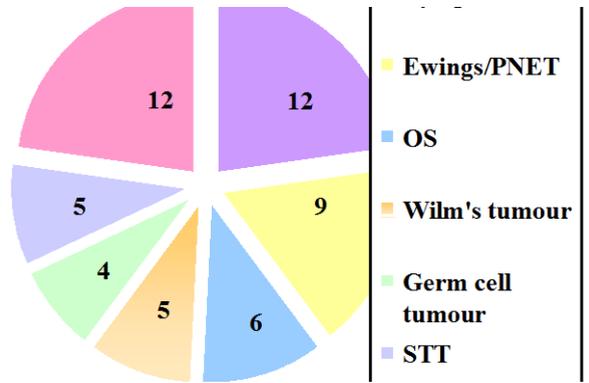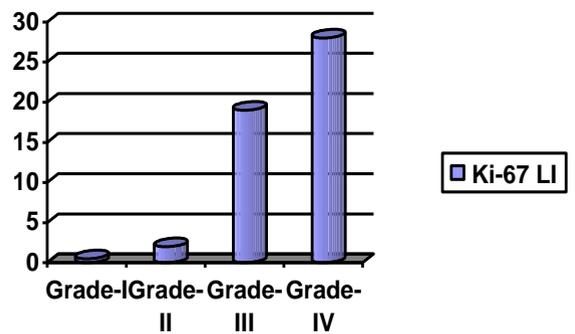**Table 3: The Ki-67 labelling index for CNS neoplasm**

Kruskal- Wallis one way Analysis of variance
Chi- Square = 17.871
Degrees of freedom = 3 P value = 0.0001

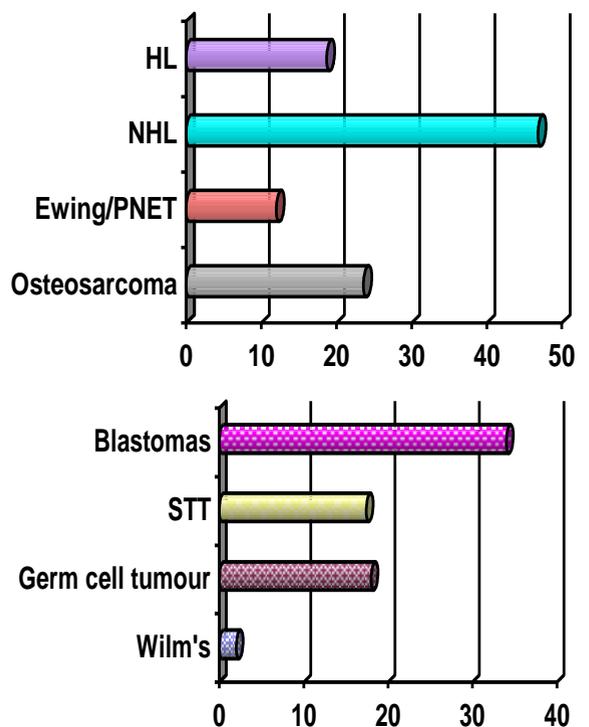**Table 4: The Ki-67 labelling index for non CNS neoplasms**

Table 1: Comparison of number of CNS cases & others

Table 2: Non CNS neoplasms

Table 5: Patient characteristics of different pediatric tumour types

Tumour	Number of cases	Male	Female	Age range
Brain tumours	22	13	9	2-17
Lymphomas	12	10	2	3-17
Ewing's/PNET	9	7	2	3-15
Osteosarcoma	6	4	2	15-17
Wilm's tumour	5	4	1	2-5
Germ cell tumour	4	1	3	1-17
Soft tissue tumours	5	3	2	2-16
Others	12	7	5	1-17

Table 6: ki-67 labelling index

Tumour	Number of cases	Minimum %	Maximum %	Mean %	Std. Error of mean	Std. Deviation
Brain tumours	22	0.5	40	14.1136	3.0598	14.3518
Lymphomas	12	12	90	37.500	7.541	26.1238
Ewing's/PNET	9	6	28	12	2.4776	7.4330
Osteosarcoma	6	10	40	23.667	4.4845	10.984
Wilm's	5	1	3	1.800	0.3741	0.8366
Germ cell	4	12	26	14.40	4.2614	9.528
STT	5	3	42	17.4	7.580	16.9499
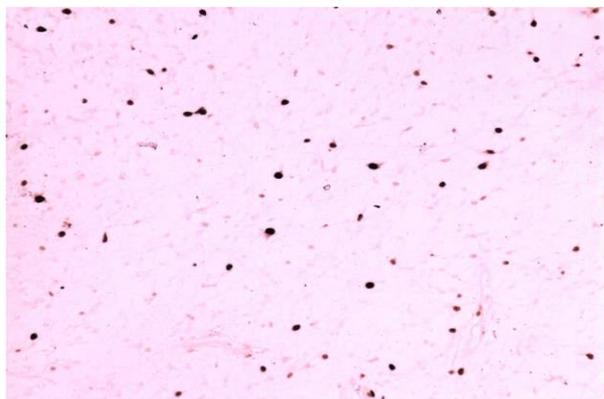
Fig. 1a: Ki-67 labelling in Grade II Glioma, x100
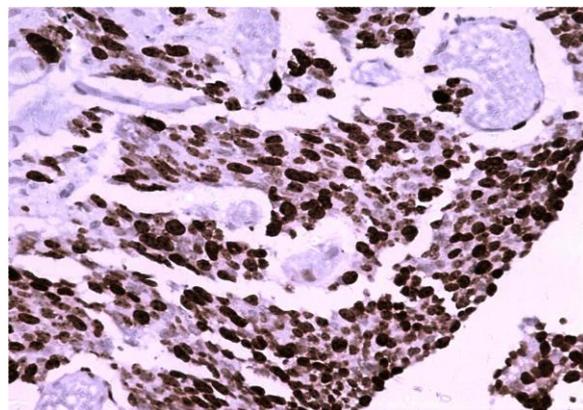
Fig. 1c: Medulloblastoma, Ki-67 labelling, x100
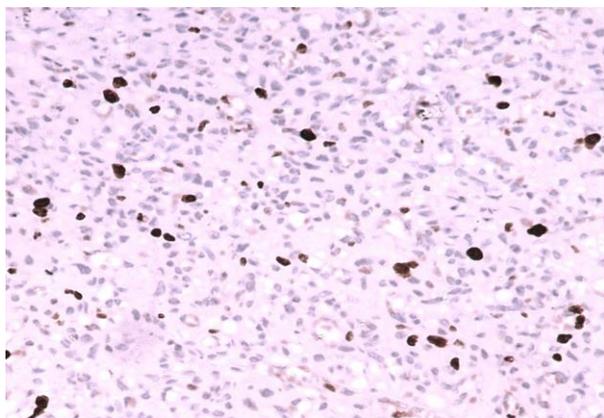
Fig. 1b: Ki-67 labelling in Grade III Glioma, x100
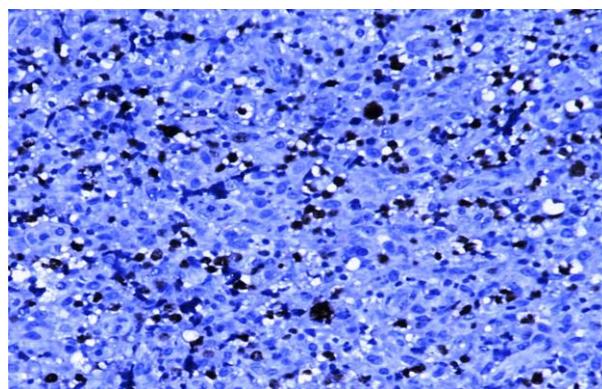
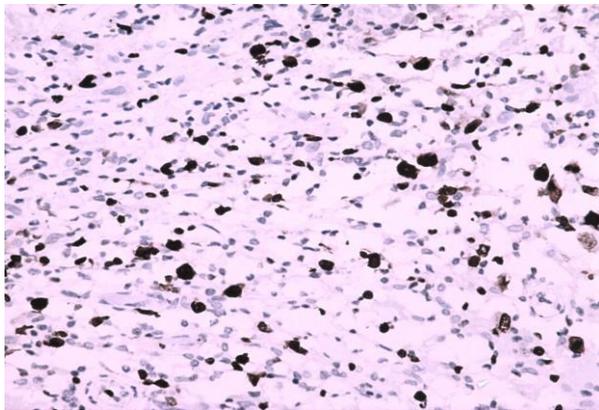**Fig. 2: Ki-67 labelling in Non Hodgkins lymphoma (DLBCL)x100**

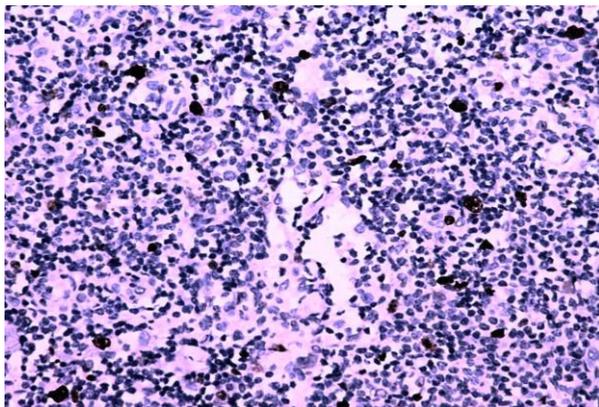**Fig. 3: Ki-67 labelling in germ cell tumour x100**

Fig. 4: Ki-67 labelling in Neuroblastoma x100

Osteosarcoma, germ cell tumour and soft tissue tumour have a moderate proliferative index while Wilm's tumour and Ewing's sarcoma have a low proliferative index. There was a statistically significant correlation between the Ki-67 labelling index and various grades of paediatric brain tumous (p <0.05). Comparative analysis of Ki-67 labelling index in all pediatric solid tumours have been depicted in Table 6.

Discussion

Pediatric solid tumours are a diverse group of lesions with an unpredictable clinical behaviour and local relapse. In our study, the most common PST's consisted of Pediatric brain tumours (31%) followed by Lymphomas (16%), Ewing's sarcoma/PNET (12%), Osteosarcoma (8%), Wilm's tumour (6%), Germ cell tumour (5%), Soft tissue tumours (6%), and Others (16%). This correlates with a study by Ries, L.A.G., Melbert D., et al[2] where the order was Brain tumours (25%), Lymphomas (10%), Neuroblastoma (8%), Wilms tumour (6%), Bone tumours (5%) and Others (44%). There was a male predominance with the male: female ratio 2.3:1, similar to a study documented by Harmon BE, Friedman K., et al[3] All cases of Wilm's tumour occurred in first decade (less than 5 yrs) while all cases of Osteosarcomas presented in the second

decade. There was no specific age prediliction in rest of the tumours.

In our study we evaluated the Ki-67 labelling index in pediatric brain tumours. The Ki-67 LI varied from <1 to 1% in Grade I gliomas with a mean of 0.8%. In Grade II neoplasms the LI varied from 1 to 4% with a mean of 2.3%. Grade III neoplasms had a range between 16 to 20% with a mean of 18%. For Grade IV the range was from 12 to 40% with a mean of 28.8% whereas in a study by Subhalakshmi Sengupta, Uttara, et al.,[1] the mean Ki-67 LI for Grade I gliomas was 0.9%, for Grade II gliomas range was between 1 to 3% and mean LI 1.57%, Grade III neoplasms ranged between 12 to 45% with a mean of 28.8%. The Ki-67 LI varied from 4 to 80% in Grade IV gliomas with a mean LI of 43.2%. On comparison, there was only a marginal difference in Ki-67 LI in Grades I & II while the mean Ki-67 labelling Index of Grade III and IV neoplasms were higher than in our study.

The growth fraction of both Hodgkin's and Non-Hodgkin's lymphomas are highly variable with the Ki-67 labelling index ranging from 12 to 90%. The mean Ki-67 LI for Hodgkin's Lymphoma was 18.7% and for Non-Hodgkin's Lymphoma 46.87%. Proliferation was highest in DLBCL which ranged from 50 to 90% which was in accordance with a study reported by Rodney R. Miles, Martine Raphael, et al.[4]

The mean proliferative index in Ewing's sarcoma/ PNET was 12% which was in concordance with a study reported by Saeed Saeedinia, Mohsen Nouri et al.,[5] where the LI ranged from 10 to 15%.The mean of Ki67 LI in low grade Osteosarcoma was 9.9% whereas in high grade Osteosarcoma the mean was 21.6% in a study by Moumita Paul, Arnab Karmakar, et al.[6] In our study the LI for Osteosarcomas ranged from 10 to 40% with a mean of 23.6%.

Wilm's tumour showed a Ki-67 labelling index ranging from 1 to 3% with a mean of 2% while reports by Mazen A. Ghanem, Theo H. Van der Kwast, et al.,[7] and Das RN, Chatterjee U, et al.,[8] revealed a high proliferative index in the epithelial (57.2%) and blastemal (39.53%) components. This may be explained by the small sample size and early stage of the tumour in our study.

In Pediatric Germ cell tumours the Ki-67 index ranged from 12 to 26% which correlates with a study by Shigeto Kawauchi, Xiu Ping Liu, et al.,[9] which showed the mean Ki-67 LI as 24.2 ± 10.1%. The Ki-67 LI for soft tissue sarcomas (predominant being rhabdomyosarcomas) ranged from 3 to 42% with a mean of 17.4%, which was in concordance with a study by San Miguel-Fraile P, Carrillo-Gijón R, et al.,[10] who evaluated a range of 0 to 45% and a of mean 18%. The mean proliferative index of other neoplasms like papillary carcinoma thyroid was 7%, blastomas 34%, skin tumours 35.3%, and neuroendocrine tumours 13%. The literature reports varying range of Ki-67 LI based on the clinical behaviour of the tumour.

We found that Ki-67 labelling index showed a varied expression in different tumours and it may throw light on the proliferative capacity of the tumor. There was a statistical significant correlation between the various grades and Ki-67 LI (p <0.05) in pediatric brain tumors which is similar to that seen in adult brain tumors.

Nuclear morphometry and evaluation of the cell proliferative activity have been reported to be useful tools in predicting prognosis of solid tumours. Assessing the presence of cell cycle related proteins yield important information about the biological behaviour of the tumour.[11] Mitotically active tumours are thought to have a poorer prognosis than inactive tumours.

Disease free survival and overall survival correlate with lower Ki-67 labelling indices. Conversely, higher Ki-67 labelling indices have been associated with decreased survival. The remaining proliferative activity in tumour tissue after chemotherapeutic treatment may be of prognostic value in predicting the clinical course of the disease after surgical removal of the tumour.[7]

Conclusion

In, conclusion, our study suggests that Ki-67 has a potential prognostic role in Pediatric solid tumours particularly in brain tumours where it correlates well with WHO Grade. Ki-67 immunostaining using the monoclonal antibody MIB-1 represents a simple, quantifiable and reproducible method to determine the tumour cell dynamics and proliferation index. This biological predictor potentially helps to identify patients with high risk of progression and recurrence and also guides for adjuvant chemotherapy or radiotherapy.

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