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Original Article

Pathology Section

Paediatric Peripheral Primitive Neuroectodermal Tumour – A Clinico-Pathological Study from Southern India

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ABSTRACT

Introduction: Primitive Neuroectodermal Tumour (PNET)/Ewing Sarcomas (ES) are aggressive childhood malignancies with neuroectodermal differentiation.

Aim: To study the clinical presentation, morphology, Immunohistochemistry (IHC), management and outcome of all the cases of paediatric pPNET/ES reported in our tertiary care centre over a period of six years.

Materials and Methods: This was a retrospective study conducted at Sri Ramachandra Medical College and Research Institute, Chennai, India. All biopsy proven cases of peripheral PNET/ES, in patients less than 18 years of age for a period of six years were included in this study. The corresponding clinical details regarding initial presentation, treatment and follow up were retrieved from the case files and analysed. Survival rate was calculated and Kaplan-Meier survival curve was plotted.

Results: We describe eleven cases of paediatric peripheral PNET/ES. The mean age at presentation was 94.08 (±58.27) months with a male/female ratio of 1.2:1. About 27.3% cases, all male with a mean age of 140 months at presentation, had distant metastasis during initial diagnosis. Biopsy showed small round blue cell morphology on light microscopy. IHC revealed strong membranous staining for CD99 in all cases. All children were treated with neo-adjuvant chemotherapy and then surgery, followed by radiotherapy if indicated. The cases were followed up for a mean duration of 20.82 months (ranging from one to 66 months). Nine children are doing well on follow up (81.8% survival rate). Two cases with metastasis at initial presentation died. Patients with metastatic disease exhibited a mean duration of survival of 9.66 (±7.24) months and those with localized disease exhibited a mean duration of survival of 25 (±22.88) months.

Conclusion: Metastasis at diagnosis is the single most important factor affecting prognosis. This was reflected in the present study where cases with metastasis exhibited a short mean duration of survival when compared to localized disease. It is likely that many cases of PNET/ES were not accurately identified in the past as IHC plays a vital role in the diagnosis of these small round blue cell tumours. IHC in adjunct with molecular studies has improved diagnostic accuracy. Multidisciplinary management and good supportive care when the lesion is localized has lead to improved survival.

Keywords: Children, CD99, Ewing sarcoma, Survival

INTRODUCTION

Peripheral PNET/ES falls under the category of PNET family of tumours. They are small round blue cell tumours with varying degrees of neuroectodermal differentiation occurring in the bone and soft tissues with common chromosomal translocations. Peripheral PNET (pPNET)/ES are quite rare and comprise 5%-10% of paediatric bone tumours and 4%-17% of paediatric soft tissue tumours [1,2]. They tend to have an aggressive clinical course with an overall three year survival rate of around 56%-65% [3]. In the past 10-15 years, the advent of IHC and advances in molecular studies has greatly enhanced the scientific knowledge regarding the tumour biology of PNET family of tumours. This has enabled improved diagnostic accuracy and treatment options with better survival outcomes.

Hence, the present study was done to evaluate the clinical presentation, morphology, IHC, management and outcome of all the cases of paediatric pPNET/ES reported in our tertiary care centre over a period of six years.

MATERIALS AND METHODS

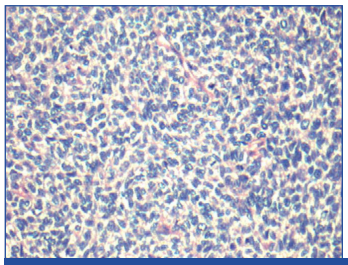
This was a record based retrospective study conducted at Sri Ramachandra Medical College and Research Institute, Chennai with Institutional Ethical Committee clearance. All biopsy proven cases of peripheral PNET/ES, in patients less than 18 years of age for a period of Jan 2010 to Dec 2015 were included in this study. A diagnosis of PNET/ES was made if the tumour cells showed diffuse, strong membranous staining for CD99. Positive controls were used. Cases with CD99 negative and those older than 18 years were excluded. The corresponding clinical details regarding initial presentation, treatment and follow up were retrieved from the case files and analysed. Survival rate was calculated and Kaplan-Meier survival curve was plotted. During the study period there were a total of 140 paediatric solid tumours of which 76 were small round blue cell tumours (54.3%). A total of 11 cases (14.6% of paediatric solid tumours) met the selection criteria and were included in our study.

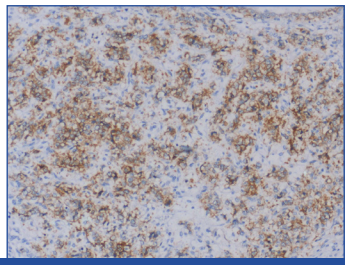
RESULTS

The clinical profile is shown in [Table/Fig-1]. The patients' ages ranged from 3 months to 17 years. The mean age at presentation was 94.08 (±58.27) months with a male/female ratio of 1.2:1 (six boys and five girls). The girls presented earlier at a mean age of 53.4 months (ranging from 3 to 72 months) whereas the boys presented at a mean age of 128 months (ranging from 48 to 204 months). The majority of the tumours (63.6%) were located in the trunk (seven cases) followed by two each in humerus and femur. Eight children presented with localized disease. Three patients, all male, (27.3% of cases) presented with metastasis at the time of

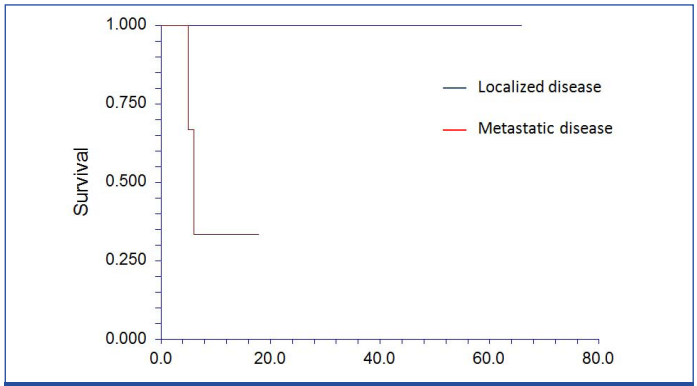
S No	Gender/ Age (years)	Primary site	Staging	Light micros-copy	Immunohis-tochemistry (Positive)	Chemo-therapy	Surgery	Radiation	Outcome	Duration of follow up (months)
1	M/12	Left 2nd rib	Localized	SRBCT	Vm, CD99	VDC & IE	Total excision of left 2nd rib	Nil	Alive and well	36
2	F/4	Dorso lumbar region D10-L2	Localized	SRBCT	Vm, CD99,S100	VDC & IE	Excision biopsy	Nil	Alive and well	48
3	F/6	Right humerus	Localized	SRBCT	Vm, CD99	VDC & IE	Local resection	Nil	Alive and well	66
4	M/11	Left femur	Metastatic (pulmonary metastasis)	SRBCT	Vm, CD99	VIDE & Topotecan	Local resection	50 Gys to primary site	Died due to relapse	6
5	M/13	Left 4th rib	Localized	SRBCT	Vm, CD99	VDC & IE	Excision of left 4th rib	54 Gys to primary site	Alive and well	1
6	M/17	Left hip joint	Metastatic (pulmonary metastasis)	SRBCT	CD99	VIDE & Topotecan	Nil	54 Gys to left pelvis and whole lung	Alive and well	18
7	F/6	Left 5th rib	Localized	SRBCT	Vm, CD99	VDC & IE	Excision of left 5th rib	50 Gys to primary site	Alive and well	15
8	M/4	Right 11th and 12th rib	Localized	SRBCT	Vm, CD99, Syn	VDC & IE	Excision of right 11th and 12th rib	50 Gys to primary site	Alive and well	18
9	F/ 3 months	Left suprascapular region	Localized	SRBCT	Vm, CD99	VDC & IE	Resection of mass	Nil	Alive and well	12
10	F/6	Abdomen	Localized	SRBCT	Vm, CD99	VDC & IE	Resection of mass	Nil	Alive and well	4
11	M/7	Left humerus	Metastatic	SRBCT	Vm, CD99	VIDE & Topotecan	Nil	Nil	Progressive disease, on palliative care, died	5

[Table/Fig-1]: Summary of clinical and histopathological details and follow up.
SRBCT – Small round blue cell tumour; Vm – Vimentin; Syn – Synaptophysin
V - Vincristine, D - Doxorubicin, C - Cyclophosphamide, I – Ifosfamide, E – Etoposide





[Table/Fig-2]: Light microscopy shows sheets of monotonous small round blue cells (Haematoxylin and Eosin, 20X). **[Table/Fig-3]:** IHC shows diffuse membranous positivity for CD99 (10X).



[Table/Fig-4]: Kaplan-Meier survival curve for localized and metastatic pPNET.

initial diagnosis.

By light microscopy the tumours showed diffuse sheets and focal nests of monotonous small round blue cells with scant cytoplasm [Table/Fig-2]. There was increased and atypical mitosis and many apoptotic bodies. The common differential diagnoses on light microscopy included lymphoma, neuroblastoma, embryonal rhabdomyosarcoma, small cell osteosarcoma and pPNET/ES based on the location and age. For every case a choice of IHC markers was made from a panel which included vimentin, CD45, CD34, CD99, desmin, myogenin, S100, BCL2, synaptophysin, chromogranin and cytokeratin. All the cases were unequivocally positive for CD99 by IHC [Table/Fig-1,3].

The children were managed with combination chemotherapy, surgery and radiation. All patients received 12 weeks of neo-adjuvant chemotherapy consisting of Vincristine, Doxorubicin and Cyclophosphamide (VDC) alternating with Ifosfamide and Etoposide (IE) or topetecan followed by resection of the tumour and adjuvant radiotherapy if required. Mean follow up duration was 20.82 months (minimum 1 month, maximum 66 months). Nine children are alive and well and are on regular follow up. Two children, who presented with metastatic disease, died at 5 months and 6 months after diagnosis due to progressive disease and relapse respectively (18.2% mortality). The Kaplan-Meier survival curve is shown in [Table/Fig-4].

DISCUSSION

The PNET family of tumours are small round cell tumours that have varying degrees of neuroectodermal differentiation. They can be classified into three groups namely Central Nervous System (CNS) PNET (arising in central nervous system), neuroblastoma (arising in autonomic nervous system) and peripheral PNET (pPNET) (arising from tissues outside the central and autonomic nervous system) [4]. Peripheral PNETs include Ewing sarcoma (osseous and extraosseous), malignant peripheral PNET or peripheral neuroepithelioma of bone and soft tissues and Askin tumour (PNET/ES of thoracopulmonary region) [5].

The PNET family of tumours is an uncommon and aggressive tumour of childhood and adolescence with an annual incidence of 2.93 per million populations [6]. The incidence rates have significantly increased over the last 25 years which may be attributed to improved diagnostic modalities [7]. Worldwide it is more common in Caucasians and less reported in the Asian population [8]. The incidence does not vary significantly according to sex but studies have reported a slight male preponderance which was also the case in our study [7,9].

Several studies have reported that pPNETs are most common in the thoracopulmonary region followed by the pelvis and extremities [3,10,11]. They are less common in the head and neck region. The site of the tumour may have a minor prognostic significance with patients having paraspinal and scapular disease faring the best, head and neck disease showing intermediate and abdominopelvic disease faring the worst [12]. In our study, the patient number was too small to conclude the effect of tumour site on survival. Both patients who died had metastatic disease at presentation hence, the effect of local site could not be determined.

It is likely that the diagnosis of PNET/ES was under-reported till the advent of IHC which enabled the diagnosis of poorly differentiated small round cell tumours. IHC profile is vital to distinguish pPNETs from other small round blue cell tumours as it affects management and prognosis. Peripheral PNETs typically express membrane positivity for CD99 which is the gene product for MIC2 [12]. It is highly sensitive but not specific for pPNET as it is frequently expressed in other small round blue cell tumours as well. However, the pattern of staining is often cytoplasmic rather than the typical membranous staining observed here [13]. pPNETs frequently co-express vimentin as well. Other nonspecific markers reported include S100, synaptophysin, neuron specific enolase and CD75 [14]. All eleven cases studied showed strong membranous CD99 and ten showed vimentin immunopositivity. One case each showed focal positivity for S100 and synaptophysin respectively. CNS PNETs lack CD99 expression [4,15,16].

Molecular studies show that all these tumours share the common reciprocal translocation of chromosomes 11 and 22. All PNET family of tumours express Friend Leukaemia virus Integration-1 (FLI-1)which is the fusion product of Ewing sarcoma gene (22q12) and FLI-1 (11q24). It is very sensitive and specific and can be detected by IHC as well as molecular methods [17,18]. Cytogenetic analysis reveals that this reciprocal translocation t(11;22) is present in approximately 85% of the cases while the remaining 5%-10% show translocation between EWS and ERG (ETS related gene) (21q22) [19,20]. Cytogenetic analysis was not done in our study taking into account the socioeconomic background of the majority of the patients.

Several studies have shown that 26%-28% of children present with distant metastasis at initial presentation [8]. This was observed in 27.3% cases in our study population as well. Reverse Transcription PCR (RT-PCR) analysis has shown that up to 30% of patients with clinically localized disease have micrometastasis in the bone marrow [21].

Current treatment recommendations advocate multimodal approach including surgical resection, adjuvant and neoadjuvant chemotherapy and radiotherapy for both localized and metastatic disease [22]. The most recommended chemotherapeutic regimen includes vincristine, doxorubicin and cyclophosphamide with ifosfamide and etoposide [23]. Complete surgical resection is vital for better survival outcomes. Long term follow up of pPNET/ES survivors treated with radiotherapy shows a six fold risk for subsequent cancers when compared to the general population [24]. This includes secondary sarcomas, acute myeloid leukaemia and myeloblastic syndromes. This appears to be radiation dose dependant and it is shown that those exposed to less than 48 Gy had no additional risk [25]. High doses of etoposide have been implicated in the incidence of secondary leukaemia reported in 1%-2% of treated cases [26]. None of our patients presented with treatment related secondary malignancies during the study period. Since it has a low incidence, a bigger study population as well as a long duration of follow up is required to comment on the same.

The most significant prognostic factor is the presence of distant metastasis [27]. In the present study, 100% cases (8/8) with localized disease were alive and well on follow up, with a mean duration of survival of 25 (± 22.88) months. Cases with metastatic disease exhibited a poor outcome with 66.7% cases (2/3) died and a short duration of survival (mean 9.66 (±7.24) months). The primary site did not have any significant effect on the outcome in our study. The Kaplan-Meier survival curve is shown in [Table/Fig-4].

LIMITATION

The study however is limited by the small number of patients as well as the short duration of follow up (mean follow up duration of 20.82 months, ranging from minimum one month to maximum 66 months).

CONCLUSION

An early accurate diagnosis of peripheral PNET is very essential as the presence of metastasis is the most important factor adversely affecting survival. This was reflected in the present study where patients with metastasis exhibited a poor outcome with (two out of three cases dead) and a short duration of survival (mean 9.66 (±7.24) months) when compared to localized disease with 100% cases alive and well on follow up. Improved diagnostic modalities including immunohistochemistry and molecular techniques have played a vital role in early diagnosis. Multidisciplinary management and good supportive care when the lesion is localized has lead to improved survival.

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