Original Article

The Role of Histopathology and Immunohistochemistry in the Diagnosis of Pediatric Round Cell Tumors: A Five Years Study at a Cancer Care Centre

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ABSTRACT

Introduction: Fletcher defines Round cell tumors as a heterogeneous, highly aggressive malignant tumors featuring primitive, small and monotonous undifferentiated morphology with increased N: C ratio. They mostly occur in children, adolescents and young adults and can occur anywhere in the body and differential diagnosis is particularly difficult due to their undifferentiated features. Increase in the burden of cancers including the round cell tumors has make it necessary for the need to diagnose accurately and also to determine the magnitude of the problem.

Aims and objective: To find out the prevalence of round cell tumor in a pediatric cancer patient coming to Dr B Borooah Cancer Institute, Guwahati, To make the list of differential diagnosis possibility based on histopathology and To see the results of immunohistochemistry.

Methods: It was a retrospective study from July2014- june2019. All cases reported as round cell tumors till the age of 18 yrs were collected from the records of pathology department. 148 cases were selected. Medical history was obtained from the patient's files: clinical examination, age at diagnosis, gender, location and histological findings. Paraffin blocks were retrieved and reviewed. Histological sections were routinely stained with hematoxylin and eosin stains. immunohistochemistry (IHC) was repeated whenever necessary.

Results: The most common pediatric round cell tumors in Dr B Borooah Cancer Institute, Guwahati is Ewing Sarcoma/PNET followed in descending order by Non Hodgkin Lymphoma > Hodgkin Lymphoma > Rhabdomyosarcoma > Neuroblastoma > Myeloid sarcoma > Neuroendocrine tumor > Wilm's tumor = Poorly differentiated > Synovial Sarcoma = Rhabdoid tumor of kidney. Male predominate female (M: F ratio = 1.2: 1) in overall round cell tumors.

Conclusions: There were significant overlap in morphology of the small round cell tumors, but careful search of the detailed history, location of tumor and presentations with imaging findings helped us to come to a probable diagnosis. Though IHC is indispensible and a very important routine accessory tool in making a diagnosis of small round cell tumor, the conclusion must be made knowing the fact that there is no antibody exclusively specific for a particular tumor entity and there could be cross-reactivity among various tumors. Interpretation of IHC results is not simply the description of positive or negative stains. Therefore, the conventional H&E morphology of the tumor in addition to the characteristics of each antibody, and the expression pattern of each targeted antigens must be considered.

Keywords: Pediatric round cell tumor. Immunohistochemistry (IHC). H &E. Ewing Sarcoma.

INTRODUCTION

Fletcher[1] defines Round cell tumors as a heterogeneous, highly aggressive malignant tumors featuring primitive, small and monotonous undifferentiated morphology with increased N: C ratio. Under such tumors, they include Ewing sarcoma, Lymphoma, Ependymomas, Wilms' tumor, Medulloblastoma, Neuroblastoma, Poorly differentiated carcinoma, Retinoblastoma, Rhabdomyosarcoma, poorly differentiated synovial sarcoma, Neuroendocrine tumor and small cell osteosarcoma.

Small round cell tumors mostly occur in children, adolescents and young adults and can occur anywhere in the body[1]. Because they are genetically and biologically different with overlap in morphology, it is necessary to identify the small round cell tumors. Moreover, increase in the burden of cancer including the round cell tumors has make it necessary for the need to diagnose accurately and also to determine the magnitude of the problem.

Differential diagnosis of small round cell tumors is particularly difficult due to their undifferentiated character[18]. For confirmation, IHC is indispensible[6,7,8]. But keeping in mind the limitations of IHC like aberrant expression of antigens and cross reactivity with other antigens, further history should be reviewed and mores investigation should be carried out wherever applicable[9]. It is important to remember that the interpretation of immunohistochemical results is not the description of positive or negative stains. The conventional H&E morphology of the tumor in addition to the characteristics of each antibody and the expression pattern of targeted antigens must be considered as well as the results of internal positive and negative controls, which may be present in tissue sections studied. A careful examination of the H&E sections, the availability of the clinical history and radiological findings help in providing a differential diagnosis and most importantly, a panel of antibodies should be employed for a conclusive diagnosis[9].

AIM AND OBJECTIVE:

The study was conducted to find out the prevalence of round cell tumors in a pediatric cancer patient coming to Dr B Borooah Cancer Institute, Guwahati. It also aim to make the list of differential diagnosis possibility based on histopathology and to see the results of immunohistochemistry,

MATERIALS AND METHODS

It was a retrospective study from July 2014- June 2019. All cases reported as round cell tumors till the age of 18yrs were collected from the records of pathology department. 148 cases were selected. Medical history were obtained from the patient's files: clinical examination, age at diagnosis, gender, location and histological findings. Paraffin blocks were retrieved and reviewed. Histological sections were routinely stained with hematoxylin and eosin stains. IHC was repeated whenever necessary.

Below are the few images of the most common tumors in our study:

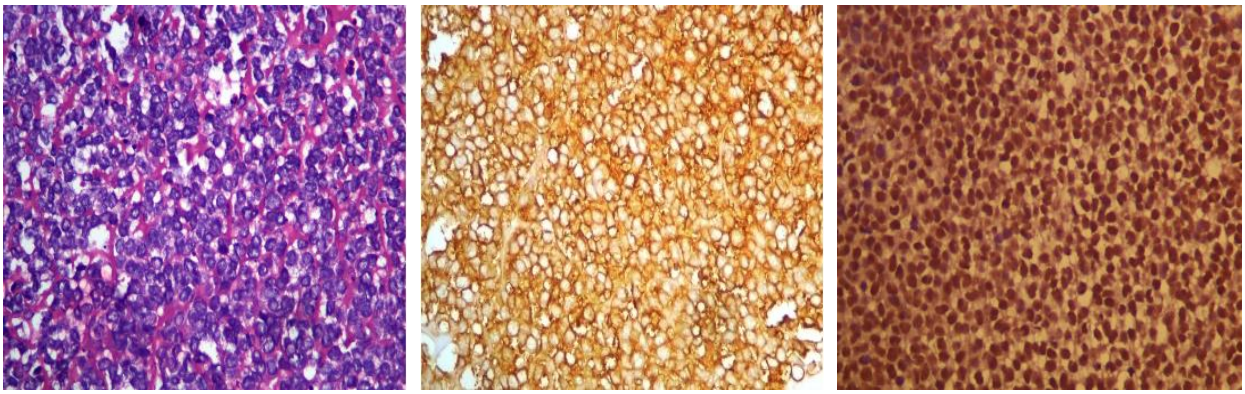

Fig 1: Ewings' Sarcoma (H&E), CD99 membranous Positivity (IHC), FLI-(IHC)

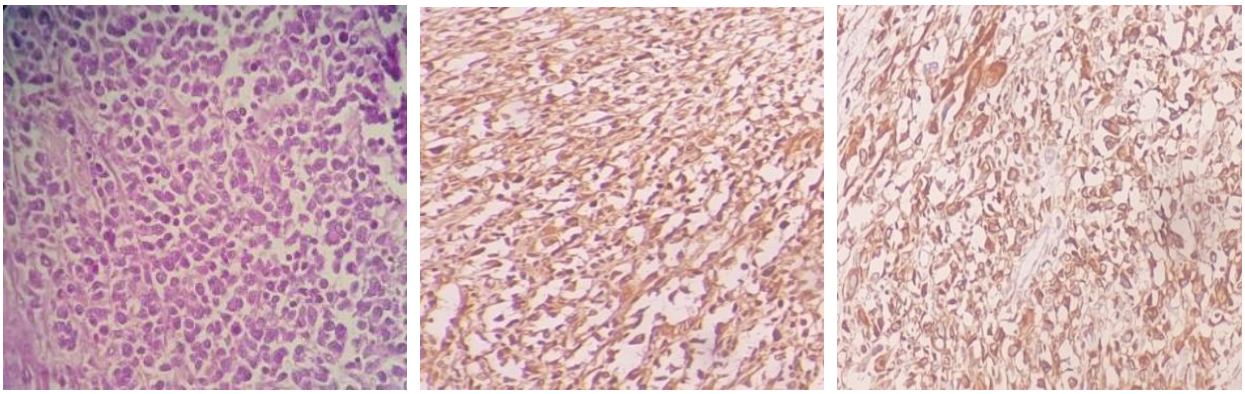

Fig 2: Rhabdomyosarcma (H&E), Myogenin (IHC), Desmin (IHC)

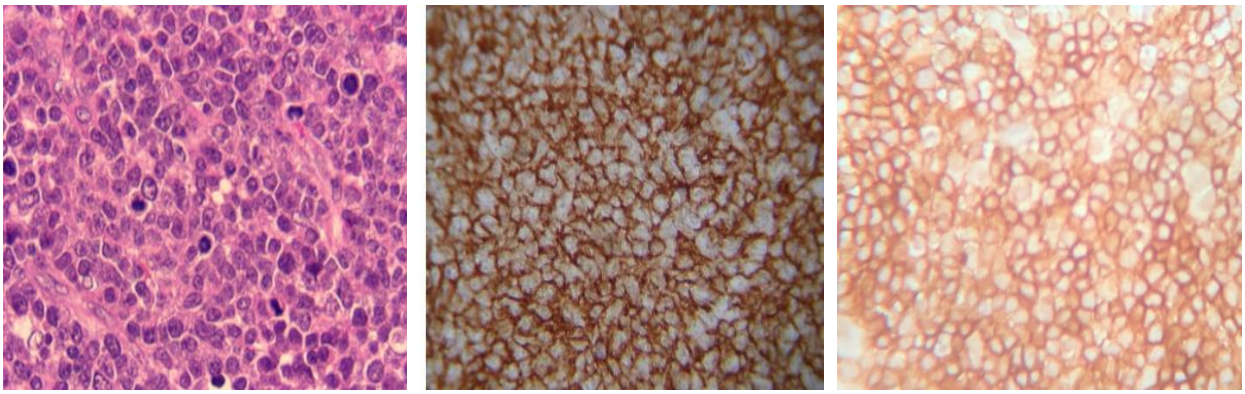

Fig 3: NHL (H&E), CD20 (IHC), CD45 (IHC)

RESULTS

Out of 148 cases, Ewing/PNET was the most common comprising of 55 cases followed by NHL (30 cases), HL(29 cases), RMS(12 cases), Neuroblastoma (09cases), Myeloid sarcoma (4 cases), Neuroendocrine tumor (03 cases), Wilm's tumor and Poorly differentiated SCC (1 each) and Synovial Sarcoma and Rhabdoid tumor of kidney (1 each).

Abbreviations used; CK-Pancytokeratin, Vim-Vimentin, Syna-Synaptophysin, Chrom-Chromogranin, Myo-Myogenin, Des-Desmin, ES/PNET-Ewing Sarcoma/Peripheral neuroectodermal Tumour, RMS- Rhabdomyosarcoma, NB-Neuroblastoma, NHL- Non Hodgkin Lymphoma, HL- Hodgkin Lymphoma, RDK- Rhabdoid Disease of Kidney, NET- Neuroendocrine Tumour, SS-Synovial Sarcoma, PDSCC-Poorly differentiated Squamous cell carcinoma, MS- Myeloid Sarcoma, Memb-Memebranous.

DISCUSSION

According to WHO[14], "a child is a person 19 years or younger unless national law defines a person to be an adult at an earlier age"

Ewing Sarcoma; 4th edition of the WHO classification of tumors of soft tissue and bone defines Ewing Sarcoma as small round cell sarcoma with characteristic molecular findings and varying degree of neuroectodermal differentiation[10]. Out of the 55 cases of ES/PNET, 47 of them were from bone (predominantly shaft of the bone) and 8 of them were from soft tissue with male predominant. The general histologic features of Ewing Sarcoma/PNET encountered in our study are mostly cells with round medium cells, vacuolated cytoplasm, fine chromatin, frequent mitoses, necrosis, rosettes seen in some case, no fibrillary matrix. All of the cases showed vimentin cytoplasmic positive, FLI-1 nuclear positive and membranous positivity for CD99. One case showed cytoplasmic positivity for both chromogranin and synaptophysin as well. Ewing Sarcoma family with neural and neuroendocrine differentiation has been widely described. [11, 12, 13.] CD 99 positivity is noted in other tumor types with cytoplasmic stain[9].

Non Hodgkin Lymphoma: NHL was the 2nd most common case encountered in our study with male predominance. Out of 30, most of them presented with generalized lymphadenopathy, 1 each from skin and sinonasal cavity turned out to be ALCL on further investigation. Morphologically, they showed diffuse Monomorphous to Polymorphous, small to large cells, folded, cleaved and grooved nuclei, pleomorphism, and high mitotic count seen in some cases, necrosis and vascular proliferation in T cell NHL. IHC showed CD45 membranous positive and CD3 and CD20 positive (Both membranous) in T cell and B cell NHL respectively. Further sub typing of all the NHL was done. CD99 (Cytoplasmic stain) and FL1-1 showed positive in 2 cases of Acute lymphoblastic lymphoma (ALL) which could be confirmed by using more panel of antibodies. Fli-1 positivity is also seen in haematopoietic malignancies and a major diagnostic pitfall in Leukocytes Common Antigen negative pediatric SRCT. [15, 16, 17.] **Hodgkin Lymphoma;** Most of the cases were from neck node with male predominance. They consisted mostly of Polymorphous lymphoid cells with occasional large cells. Large binucleated and mononucleated RS cells were also seen in some cases with background eosinophils. CD 15 and CD 30 showed cytoplasmic positivity in large RS cells. **Rhabdomyosarcoma;** 4th edition of the WHO classification of tumours of soft tissue and bone recognizes 4 major types of RMS as; 1. Embryonal RMS, 2.alveolar RMS, 3.Pleomorphic RMS and 4. Spindle cell/Sclerosis RMS.[10.] Of the 12 cases, 7 of them were from soft tissues of varying locations whereas, 5 of them were from pelvis with male predominance. Our study showed mostly sheet or alveolar pattern; Solid sheets consisted of high grade round cells. Few case show alveolar pattern with nests of tumor cells separated by fibrovascular septa which are mostly incomplete. Occasional mitoses noted. IHC showed tumor cells positive for Myogenin (nuclear) and cytoplasmic positive for both Desmin and Vimentin . CD99 showed cytoplasmic positivity in one of the case, which would have pose a diagnostic difficulty had we not employed multiple antibody panels. Few cases of CD99 expression is seen in RMS[20]. **Neuroblastoma;** Out of 9 cases, 6 presented as abdominal mass and 1 each from nasal cavity, orbit and neck node with male predominance. Morphology showed cells in lobular pattern with Salt-and-pepper chromatin, small to obvious nucleoli, neurofibrillary matrix present forming pseudo rosettes. True rosettes were also seen. IHC showed cytoplasmic positivity for both Synaptophysin and Chromogranin and negative for CD99, CD45, Desmin. **Myeloid sarcoma;** All 4 cases had different sites; 1 each from leg, orbit, node and Retro-peritoneum with equal sex ratio. Morphology from 2 cases showed cells with moderate to abundant cytoplasm, round to oval irregularly folded nuclei, distinct and prominent nucleoli, While morphology of other 2 cases showed small cells with increased N:C ratio. IHC showed cytoplasmic positivity for CD45 and MPO. CD99 showed cytoplasmic positivity in 3 of them, CD117 was both membranous and cytoplasmic positive in 1 of the case, BCL2 positive in one of the case and FLI-1 positive in 2 cases. **Neuroendocrine tumor (NET);** the data specific to pediatric **NET** epidemiology are limited due to its rare occurrence in this population. Out of 3 cases, 2 of them were from the node while 1 of them is from appendix. They consisted of cells with small size with high nuclear:cytoplasmic ratio, crushed artifact, nuclear molding, moderate pleomorphism and inconspicuous nucleoli. IHC showed cytoplasmic positivity for Synaptophysin and Chromogranin. **Poorly differentiated carcinoma;** Poorly differentiated non keratinizing variant of head and neck tumor though a rare entity in pediatric population may exhibit histologic features that overlap with that of Small round blue cell tumors[5] . We had 2 cases, 1 each from sinonasal and nasopharynx, both of them were female. They show round to polymorphic undifferentiated cells with solid growth, inconspicuous nucleoli and high mitotic count with area of necrosis. Both of the cases showed cytoplasmic positivity for pan CK and were negative for other markers. **Wilms' tumor;** There were 2 cases of wilms' tumor, both of them female. Though knowing the site and age of the patient may lead to a straight forward diagnosis, We received scant biopsied sampled and sections consisted of small undifferentiated , densely packed blue cells with scant cytoplasm, overlapped nuclei and finely dispersed chromatin. Both of the cases showed positive for WT1 (nuclear positive) and CD99 (cytoplasmic). One had focal desmin positivity. Typically Wilms tumor is composed of mixtures of blastemal, epithelium and stromal. However not all are triphasic. Tumor with more blastemal component may resemble other SRBCT[1]. **Poorly differentiated Synovial sarcoma;** We had one female patient with chest

wall tumor. Histo-morphology showed high cellularity, round cells with hyperchromatic nuclei and inconspicuous nucleoli. It was positive for CD99, BCL2 and Vimentin. **Rhabdoid tumor of kidney;** It is a rare aggressive cancer occurring in infancy and early childhood, was recognized as a distinct tumor type, although initially it was classified as a possible Rhabdomyosarcomatoid variant of wilms' tumor. Subsequent studies confirmed its distinctive nature and its designation was shortened to rhabdoid [2, 3 ,4].We had 1 male baby with tumor of kidney. Sections showed solid sheets of medium to large cells with eosinophillic cytoplasm, prominenet nucleoli. IHC showed EMA and Vimentin positive. S100 was focally positive.

The separation of the small round cell tumors is critical as management protocol varies. We noticed considerable cross-reactivity among various tumors and antibodies in our studies. So keeping in mind the limitations of IHC, like aberrant expression of antigens and cross reactivity with other antigens, further history should be reviewed and mores investigation should be carried out wherever applicable

Furthermore, the absence of antigen expression does not rule out the diagnosis. Therefore, cytogenetic and molecular genetics diagnosis now plays an important role. Many types of soft tissue sarcoma and lymphoma particularly the pediatric tumors are characterized by distinctive cytogenetic aberrations, most often reciprocal chromosome translocations, which are relatively tumor specific and thus diagnostically useful. Few examples; Several partner genes fused to EWSR1 have been identified in Ewing sarcoma, the most common is t(11;22) (q24;q12), which results in the formation of the fusion gene EWSR1–FLI1[1, 19]. Alveolar RMS carries in most cases a reproducible tumor-specific chromosome translocation, t(2;13) (q35;q14), resulting in PAX3–FOXO1 fusion [1 ,19]

The t(X;18) which fuses SYT (SS18) with SSX1, SSX2 or rarely ssx4 is characteristic of the synovial sarcoma.

Specific chromosomal translocations have been identified in some lymphoma types. They have become instrumental in shaping the classification of lymphomas, and their detection can aid in diagnosis. Amplification of the MYCN oncogene is present in increased copy number in approximately 25% to 35% of neuroblastomas and has been associated with advanced-stage tumors and more aggressive behavior [1].

Therefore, understanding of Cytogenetics and Molecular Genetic studies of these tumors are valuable to help clinicians determine further management.

CONCLUSIONS

The most common pediatric round cell tumors in Dr B Borooah Cancer Institute, Guwahati is Ewing Sarcoma/PNET followed in descending order by Non Hodgkin Lymphoma > Hodgkin Lymphoma > Rhabdomyosarcoma > Neuroblastoma > Myeloid sarcoma > Neuroendocrine tumor > Wilm's tumor = Poorly differentiated > Synovial Sarcoma = Rhabdoid tumor of kidney. Male predominate female (M: F ratio = 1.2: 1) in overall round cell tumors.

There were significant overlap in morphology of the small round cell tumors, but careful search of the detailed history, location of tumor and presentations with imaging findings helped us to come to a probable diagnosis and plan which panel of markers to put up. Further for confirmation, Immunohistochemistry was indispensible. The immunohistochemical conclusion must be made knowing the fact that there is no antibody exclusively specific for a certain tissue type or particular tumor entity. Our study had its own share of limitations. Many cases with histologically diagnosed Round Cell Tumors were excluded due to failure of the patient for follow-up. Therefore, the prevalence and burden of the case may be less than the actual scenario.

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