Original Article

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Immunohistochemical Analysis of Paediatric Small Round Blue Cell Tumours at a Tertiary Care Centre, Ludhiana, India

Pathology Section

SHIKHA NARANG[1], ARNAV KUMAR ROYCHOUDHURY[2], GUNJAN BALA[3], SUMIT DHURIA[4], PAVNEET KAUR SELHI[5]

ABSTRACT

Introduction: Small, undifferentiated cells with high nuclear-to-cytoplasmic ratios predominate in Small Round Blue Cell Tumours (SRBCT) and are characterised by their monotony. The classification of small round-cell tumours is further facilitated by Immunohistochemistry (IHC). Determine the line of differentiation using IHC, which also acts as a proxy for underlying molecular genetic changes. To identify the presence of a particular protein marker that can help with accurate cancer categorisation and diagnosis, histology uses IHC. In light of this, SRBCT are a subgroup of highly aggressive malignant neoplasms that are primarily comprised of monotonous, small, undifferentiated cells with high nuclear-to-cytoplasmic ratios.

Aim: The study is aimed to analyse the role of IHC in the SRBCT to differentiate and accurately diagnose the tumour cells using molecular markers.

Materials and Methods: This ambispective cohort study was conducted from August 1st, 2010 to 31st July 2015, a total of five years in the Pathology Department of Dayanand Medical College and Hospital (DMCH), Ludhiana, Punjab, India. All specimens of SRBCT less than 18 years of age were analysed grossly and microscopically. The study covered patients who were in the paediatric age range. The SRBCT were distinguished and categorised using immunohistochemical staining. CD99, CD20, CD15, CD30, CD3, desmin, CD45/LCA (the Lymphocyte Common Antigen), chromogranin, Myogenin, Synaptophysin (SYP), Cytokeratin (CK), and Epithelial Membrane Antigen (EMA) were among the immunomarkers used in this investigation. The results were presented using percentage and frequency statistics.

Results: Total 54 cases of SRBCT were analysed. This included 12 cases of Non Hodgkin's Lymphoma (NHL), 10 cases of Ewing's/Primitive Neuroectodermal Tumours (PNETs), 12 cases of Hodgkin's Lymphoma (HL), nine cases of rhabdomyosarcoma, four cases of neuroblastoma, two cases each of Langerhans Cell Histiocytosis (LCH) and synovial sarcoma, and one case each of olfactory neuroblastoma, sarcoma and ganglioneuroblastoma.

Conclusion: The present study shows that utilising IHC in challenging circumstances is incredibly helpful and crucial where clinical-histomorphological findings are not sufficient for arriving at the final diagnosis. The majority of SRBCT developed in younger age groups, with lymphoma being the most prevalent type.

Keywords: Cluster of differentiation, Immunohistochemistry, Non-Hodgkins lymphoma, Protein markers

INTRODUCTION

Small, monotonous, undifferentiated cells with high nuclear to cytoplasmic ratios and small, round, blue cell tumours are a subset of highly aggressive malignant neoplasms. A definitive diagnosis might be challenging because, even though traditional histological findings are typically highly predictive of tumour type, these tumours occasionally may be difficult to identify by Light Microscopy (LM) [1]. The importance of making an accurate diagnosis of paediatric small-round-cell cancers has increased as various therapeutic modalities are employed for various tumour types. It has also become crucial to further categorise tumours using IHC, as a treatment for many paediatric tumours is personalised based on patient risk.

Neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma, acute lymphoblastic leukaemia/lymphoma, and the blastematous component of Wilms' tumour can be grouped as the SRBCT of childhood [2]. While it is often relatively straightforward to arrive at a diagnosis on clinical and conventional pathological information, in a small proportion of cases this is not the case. Because it uses a specific antigen-antibody reaction to specifically visualise the distribution and amount of a specific molecule in the tissue, IHC is a crucial auxiliary technique for pathologists [3]. The utilisation of IHC has recently exploded as more and more molecules involved in illness diagnosis, treatment, and causation are found. The fact that IHC is carried out without destroying the histologic architecture, sets it apart from many other laboratory studies. As a result, it is possible to evaluate a molecule's expression pattern in a setting of the environment [4]. Dhingra H et al., involved a 5-year retrospective review of all solid tumours in children under the age of 18 [5]. The pattern of paediatric tumours was portrayed in this institution-based investigation. The neuropathological spectrum of dura-based non meningiothelial lesions identified over five years in the tertiary care centre was examined by Rao S et al., [6]. Yadav M et al., final diagnoses made using an analysis of eosin-stained tissue sections and Formalin-Fixed Paraffin-Embedded (FFPE) haematoxylin [7]. Barwad A et al., report histological analysis using immunofluorescence and electron microscopy frequently leads to an accurate diagnosis and encourages crucial treatment [8]. To diagnose primary malignant mixed Mullerian tumours, Agrawal R et al., investigated the diagnostic utility of immunocytochemistry {Malignant Mixed Mullerian Tumour (MMMT)} and Fine Needle Aspiration Cytology (FNAC) [9]. The pathologist should meticulously match the radiological, morphological, and clinical findings with a panel of IHC markers because no antibody is specific to a particular type of cancer [10]. Consequently, to differentiate and accurately diagnose the tumour cells, IHC and molecular markers are studied in this article.

Many different kinds of soft tissue tumours lack distinguishing morphological characteristics and have a hazy differentiation line. In histology, IHC is employed for a particular protein marker which can facilitate precise tumour diagnosis and classification. As a consequence, SRBCT are a kind of extremely aggressive malignant neoplasms that are primarily characterised by small,

monotonous, undifferentiated cells with high nuclear-to-cytoplasmic ratios. This group includes Ewing's/PNETs, neuroblastoma, NHL, rhabdomyosarcoma, monomorphic, LCH, synovial sarcoma, olfactory neuroblastoma, Anaplastic Large Cell Lymphoma (ALCL), ganglioneuroblastoma, and desmoplastic SRBCT, small-cell carcinoma, mesenchymal chondrosarcoma, and small-cell osteosarcoma. Due to their near histological similarities, a variety of overlapping characteristics, and a lack of distinguishing characteristics on Haematoxylin and Eosin (H&E) sections, most of the SRBCT are extremely difficult to diagnose thereby necessitating IHC and molecular studies as additional support for proper diagnosis and accurate categorisation of these tumours. Treatment and the prognosis vary greatly among these tumours, thereby necessitating an accurate diagnosis, hence making the appropriate usage of IHC even more important in these smudgy greyish scenarios [11-13]. The main goal is to examine the histological range of SRBCT during five years at a tertiary care facility, as well as the use of IHC in the identification of these cancers. Hence, the present study was conducted with aim to analyse the role of IHC markers for appropriate diagnosis to enable the clinician to give the most effective targeted and tailored treatment suited for that particular patient.

MATERIALS AND METHODS

This ambispective cohort study was conducted from 1st August 2010 to 31st July 2015 comprising of 1.5 years prospective from 1st August 2010 to 31st January 2012 and 3.5 years of retrospective analysis from 1st February 2012 to 31st July 2015, in the Pathology Department of Dayanand Medical College and Hospital (DMCH), Ludhiana, Punjab, India (Approved by the Institutional Research and Ethics Committee in the meeting held on 26.12.2013 vide letter no DMCH/TCM/2013 dated 28.12.2013). From August 2013 to August 2015, 54 patients participated in the present study at the DMCH tertiary care Hospital.

Inclusion and Exclusion criteria: The study covered patients who were in the paediatric age range. The study did not include the small, spherical, blue-cell tumours of the bone marrow and all specimens of SRBCT less than 18 years of age were analysed. The study did not include the small, spherical, blue-cell tumours of the bone marrow.

Study Procedure

Utilising the appropriate panel of immunohistochemical antibodies and the streptavidin-biotin detection method, immunohistochemical studies were carried out to classify the tumours. From the institute's pathology department's records, all cases of round cell tumours reported from bone, soft tissue, and solid organ regions were gathered. All specimens of tumours less than 18 years of age were analysed grossly and microscopically. The files contained FFPE sections of cancers identified as SRBCT on resected tissues and small biopsies. Two impartial expert observers removed and examined the paraffin blocks.

The EnVision method was used to carry out the IHC. There was no need to dilute any of the antibodies. The samples were fixed in 10% neutral formaldehyde, routinely embedded in paraffin, and cut into 4 m slices, endogenous peroxidase activity was suppressed with 0.3% Hydrogen Peroxide (H_2O_2) after being deparaffinised, microwaved for 10 minutes in citrate buffer (pH 6.0), and rehydrated in graduated series of ethanol. In an automated staining system (Agilent, America), the tissues are administered using standardised procedures. The study performed the initial blocks of IHCs stated as SRBCT following the departmental protocol using the following antibodies- Cluster of Differentiation (CD)99, Epithelial Membrane Antigen (EMA), CD45, Cytokeratin (CK), Friend Leukaemia Integration (FLI)-1, S-100, Chromogranin, Vimentin, Desmin, Synaptophysin (SYP) and Myogenin. To distinguish and classify SRBCT, further IHC stains were performed. The lymphocyte common antigen (CD45/LCA), CD30, CD99, CD20, CD15, and CD3 (called Monoclonal antibodies directed against the E2 protein (MIC2) or cluster of differentiation 99), Myogenin, desmin, CK, SYP, chromogranin, S100 were the immunomarkers used in this investigation. The statistical analysis of CD15 and CD30 has been included in the study as seen in [Table/Fig-1]. The membranous/cytoplasmic/nuclear staining of various IHC markers was studied under Light Microscopy (LM).

Diagnosis	Sites (cases distribution)	Nuclear feature and initial diagnosis	Immunohistochemistry (IHC) findings
RMS	Trunk (9 cases)	Round hyperchromatic, RMS	Positive marker: myogenin, Negative marker: CK, LCA
HL	Lymph node (12 cases)	Round vesicular nucleus, HL	Positive marker: CD 15, CD 30, Negative marker: CD3, CD20
NHL, B cell type	Nasopharynx (12 cases)	Round vesicular nucleus, carcinoma or lymphoma	Positive marker: CD45, CD20, Negative marker: CD3, CK
HL	Lymph node (12 cases)	Round vesicular, lymphoma	Positive marker: CD 15, CD 30, Negative marker: CD3, CD20
ALCL, ALK Positive	Lymph node (5 cases)	Round vesicular, lymphoma	Positive marker: CD45, CD3, ALK, Negative marker: CD15, CD30, CK, Vimentin
HL	LN (12 cases)	Round vesicular, lymphoma	Positive marker: CD 15, CD 30; Negative marker: CD3, CD20
RMS	Trunk (9 cases)	Oval, malignancy	Positive marker: myogenin, Vimentin; Negative marker: CK, LCA
RMS	Head n neck (9 cases)	Round hyperchromatic, MRCT	Positive marker: myogenin; Negative marker: CK, LCA
NHL	Retroperitoneum (12 cases)	Round hyperchromatic, MRCT	Positive marker: Cd45, Vimentin; Negative marker: CK, myogenin
HL	LN (12 cases)	Round hyperchromatic, MRCT	Positive marker: CD 15, CD 30; Negative marker: CD3, CD20
LCH	Bone (2 cases)	Round vesicular, Lymphoma	Positive marker: CD1a, S100; Negative marker: CD45
ES	Flat bone (10 cases)	Round vesicular with grooves, LCH	Positive marker: Vimentin, CD99; Negative marker: CK, CD45
ES	Long bone (10 cases)	Round hyperchromatic, MRCT	Positive marker: CD99, MIC2; Negative marker: CD45
Sarcoma	Head, neck (1 case)	Round, MRCT	Positive marker: Vimentin; Negative marker: CK, LCA
HL	LN (12 cases)	Round vesicular, malignancy	Positive marker: CD 15, CD 30; Negative marker: CD3, CD20
HL	LN (12 cases)	Round vesicular, lymphoma	Positive marker: CD 15, CD 30; Negative marker: CD3, CD20
HL	LN (12 cases)	Round vesicular, lymphoma	Positive marker: CD 15, CD 30; Negative marker: CD3, CD20
RMS	H&N (9 cases)	Round vesicular, lymphoma	Positive marker: myogenin, Vimentin; Negative marker: CK, LCA
Neuroblastoma	Adrenal (4 cases)	Round pleomorphic, MRCT	Positive marker: NSE, chromogranin; Negative marker: CK, myogenin
NHL	Nasopharynx (12 cases)	Round hyperchromatic, MRCT	Positive marker: Vimentin, CD45; Negative marker: CD20
NHL T cell lymphoblastic lymphoma	LN (12 cases)	Round hyperchromatic, MRCT	Positive marker: CD45, CD3, CD34; negative Marker: CK, LCA

RMS	H&N (9 cases)	Round hyperchromatic, MRCT	Positive marker: myogenin, Vimentin; Negative marker: CK, myogenin
NHL	LN (12 cases)	Round hyperchromatic, MRCT	Positive marker: Vimentin, CD45; Negative marker: CD45, myogenin, CK
ES/PNET	Trunk (10 cases)	Round vesicular, NHL	Positive marker: Vimentin, CD99; Negative marker: CD20
NHL T cell lymphoblastic lymphoma	LN (12 cases)	Round pleomorphic, MRCT	Positive marker: CD3, CD45, CD34; Negative marker: CD45, CD3
NHL T cell lymphoblastic lymphoma,	LN (12 cases)	Round hyperchromatic, NHL	Positive marker: CD20, CD34; Negative marker: CK, Vimentin, CD45
Neuroblastoma	Retroperitoneum (4 cases)	Round hyperchromatic, NHL	Positive marker: chromogranin; Negative marker: CD45, CD3
NHL B cell lymphoblastic lymphoma,	LN (12 cases)	Round hyperchromatic, MRCT	Positive marker: CD20, CD34; Negative marker: CD3, CD20
HL	LN (12 cases)	Round hyperchromatic, NHL	Positive marker: CD 15, CD 30; Negative marker: CD45, CK, myogenin
ES	Flat bone (10 cases)	Round vesicular, HL	Positive marker: CD99, Vimentin; Negative marker: CD45, CK, myogenin
ES	Long bone (10 cases)	Round hyperchromatic, MRCT	Positive marker: CD99, Vimentin; Negative marker: CD45, Vimentin
Neuroblastoma	Adrenal (4 cases)	Round hyperchromatic, MRCT	Positive marker: chromogranin; Negative marker: CD45, CK
ES/PNET, MIC 2 Positive	Trunk (10 cases)	Round hyperchromatic, MRCT	Positive marker: CD99, CD34, Vimentin, MIC2; Negative marker: CD20
NHL T cell lymphoblastic lymphoma	Mediastinum (12 cases)	Round hyperchromatic, ES	Positive marker: CD3, CD34, CD45; Negative marker: CD3, CD20, ALK
HL	LN (12 cases)	Round, NHL	Positive marker: CD 15, CD 30; Negative marker: Desmin, S100, SMA
Synovial sarcoma, monophasic	H&N (2 cases)	Round vesicular, HL	Positive marker: Vimentin, BCL-2, CD31; Negative marker: CK, LCA
RMS	LL (9 cases)	Oval to spindle, MMT	Positive marker: Myogenin, Vimentin; Negative marker: CD3, CK
NHL- Burkitt lymphoma,	Ileum (12 cases)	Round MRCT	Positive marker: CD45, CD20, Bcl-6, CD10; Negative marker: CD45, Vimentin, CK, Myogenin
Olfactory neuroblastoma	Nasopharynx (1 cases)	Round vesicular, NHL	Positive marker: Synaptophysin; Negative marker: CK, CD45, Myogenin
ES	Short bone (10 cases)	Round hyperchromatic, MRCT	Positive marker: Vimentin, CD99; Negative marker: CD3, CD20
HL	LN (12 cases)	Round hyperchromatic, ES	Positive marker: CD 15, CD 30; Negative marker: CK, CD45
ES	Long bone (10 cases)	Round vesicular, HL	Positive marker: Vimentin, CD99; Negative marker: CK, LCA, SMA
RMS	Trunk (9 cases)	Round hyperchromatic, MRCT	Positive marker: Myogenin, Vimentin; Negative marker: CD3
NHL-Burkitt lymphoma	Ileum (6 cases)	Oval, MRCT	Positive marker: CD45, CD20, CD 10, Bcl-6; Negative marker: CD45, Myogenin, Synaptophysin
ES,	Flat bone (10 cases)	Round vesicular, NHL	Positive marker: CD99, FLI-1; Negative marker: CK, Myogenin
ES	Flat bone (10 cases)	Round hyperchromatic, MRCT	Positive marker: Vimentin, CD99, MIC2; Negative marker: CD3, CD20
HL	LN (12 cases)	Round hyperchromatic, MRCT	Positive marker: CD 15, CD 30; Negative marker: SMA, CD34, S100, Desmin
Synovial sarcoma monophasic,	LL (2 cases)	Round Vesicular, HL	Positive marker: Vimentin, Bcl-2; Negative marker: CK, LCA
RMS	Trunk (9 cases)	Oval to Spindle, MMT	Positive marker: Myogenin, Vimentin; Negative marker: CD45
LCH	Bone (2 cases)	Trunk, MRCT	Positive marker: S100, CD1a; Negative marker: CD99, CD45
Ganglioneuroblastoma, NSE Pos	Tetroperineum (1 cases)	Bone, LCH	Positive marker: S100, Synaptophysin, Chromogranin; Negative marker: CD45, Vimentin
Neuroblastoma	Mediastinum (4 cases)	Retroperitoneum, Ganglioneuroblastoma	Positive marker: Chromogranin; Negative marker: CD3, CD20
HL	LN (12 cases)	Round hyperchromatic, MRCT	Positive marker: CD 15, CD 30.
RMS	H&N (9 cases)	Round hyperchromatic, MRCT	Positive marker: Myogenin, Vimentin; Negative marker: CK, LCA

[Table/Fig-1]: Cases distribution with immunohistochemical analysis.
CD: Cluster differentiation; HL:Hodgkin's lymphoma; PNET: Primitive neuroectodermal tumour; ES: Ewing's sarcoma; RMS: Rhabdomyosarcoma; NHL: Non Hodgkin's lymphoma; NB: Neuroblastoma; LL: Laminated layer; LN: Lymph nodes; LCH: Langerhans cell histiocytosis; ALCL: Anaplastic large cell lymphoma; MMT: Malignant mesenchymal tumour; ALK positive:anaplastic lymphoma kinase positive; MIC2: positive-single-chain type-1 glycoprotein; MRCT: Malignant Round cell tumours; SMA: smooth muscle actin; BCL2: B-cell leukaemia/lymphoma 2 protein; LCA: Leucocyte common antigen; CK: Cytokeratin

STATISTICAL ANALYSIS

The results were presented using percentage and frequency statistics.

RESULTS

The SRBCT were found in 54 cases, and the results of the study are shown in [Table/Fig-2]. In the present study, there were 12 cases (22.2%) of NHL and 12 (22.2%) of HL cases, and the final diagnosis showed that 10 people have the ES/PNET tumour (18.5%). Moreover, the least cases were diagnosed as Neuroblastoma (7.4%), Synovial sarcoma and monophasic (3.7%), LCH (3.7%), Olfactory neuroblastoma (1.9%), Ganglioneuroblastoma (1.9%) and Sarcoma (1.9%).

[Table/Fig-3] shows the cases' age distribution for both males and females. Patients with small rounded blue cell tumours ranged in age from one year to 18 years in the current study. The age range from 11≤15 years exhibited the highest percentage of tumour cases

Final diagnosis	Number of cases (%)
NHL	12 (22.2%)
HL	12 (22.2%)
ES/PNET	10 (18.5%)
Rhabdomyosarcoma	9 (16.6%)
Neuroblastoma	04 (7.4%)
Synovial sarcoma, monophasic	2 (3.7%)
LCH	2 (3.7%)
Olfactory neuroblastoma	1 (1.9%)
Ganglioneuroblastoma	1 (1.9%)
Sarcoma	1 (1.9%)
Total	54 (100%)

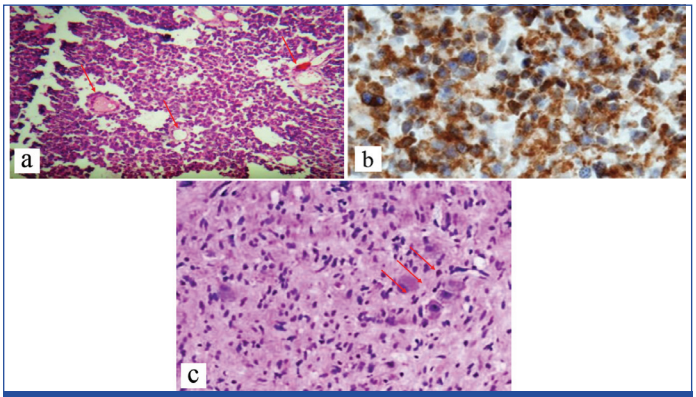
[Table/Fig-2]: Incidence of Small Round Blue Cell Tumours (SRBCT).
NHL: Non Hodgkin's lymphoma, HL: Hodgkin's lymphoma, ES/PNET: Ewing's sarcoma/primitive neuroectodermal tumour, LCH: Langerhans cell histiocytosis

(18 cases, 33.3%). The least number of tumour cases occurred in the age group 6≤10 years (9 cases, 16.6%). In the present study, the tumour has mostly affected males with a high range of (40 cases, (74.1%)) when compared to females 14 (25.9%) cases. [Table/Fig-1] illustrates the demographic details of age and sex of 54 cases with the predicted nuclear features.

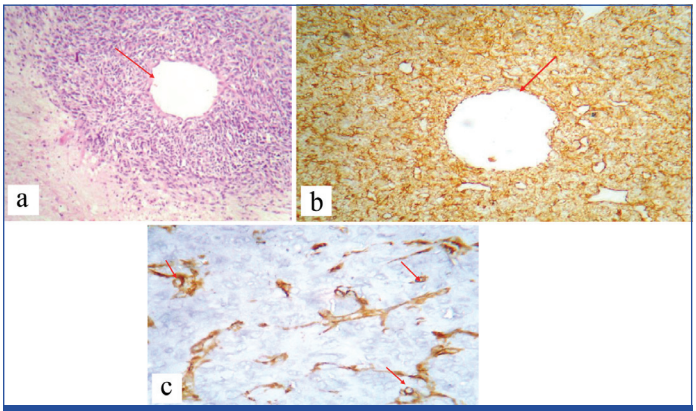
0-5 years		6-10 years		11-15 years		16-<18 years	
Male	Female	Male	Female	Male	Female	Male	Female
10	2	8	1	12	6	10	5

[Table/Fig-3]: Age and sex-wise distribution of Small Round Blue Cell Tumours (SRBCT).

On Histochemical analysis, Neuroblastoma showed sheets of small round cells and perivascular rosettes [Table/Fig-4(a)]. The cases presented areas with fibrillary material, differentiated areas often displayed a strong immunoreactivity, whereas undifferentiated areas with excessive cellularity were less positive [Table/Fig-4(b)]. The ganglioneuroblastoma results revealed an abundant neuroblastic component that was dispersed widely or rosette-like and a clear ganglionic maturation with big, neurons with a high degree of differentiation [Table/Fig-4(c)]. [Table/Fig-5a-c] showed IHC analysis of Monophasic Synovial Sarcoma while various presentations of rhabdomyosarcoma. [Table/Fig-6a,b] also depicted characterstic features.

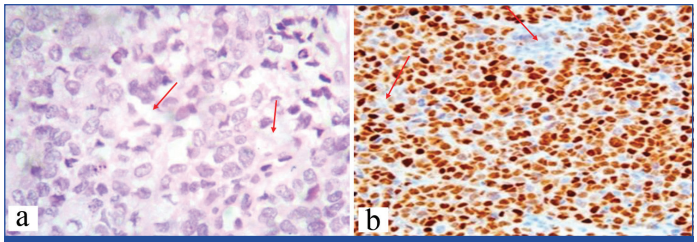


[Table/Fig-4]: Photomicrograph of Neuroblastoma. (a) Photomicrograph of Neuroblastoma showing sheets of small round cells and perivascular rosettes (H&E, X100). (b) Photomicrograph of neuroblastoma showing synaptophysin (SYP) positivity (IHC, X400). (c) Photomicrograph of ganglioneuroblastoma showing ganglion cells (H&E, X400).



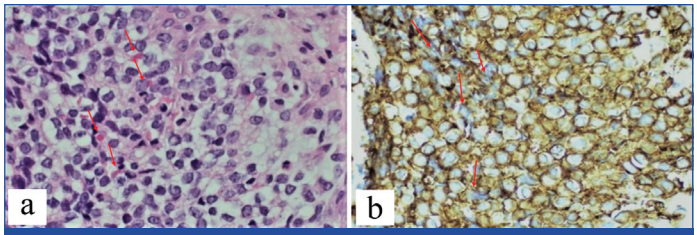
[Table/Fig-5]: Photomicrograph of monophasic synovial sarcoma. (a) Photomicrography of monophasic synovial sarcoma showing oval cells arranged in alternating hypocellular and cellular areas with haemangiopericytoma focus (H&E, X100). (b) Photomicrography of monophasic synovial sarcoma showing vimentin cytoplasmic positivity in tumour cells (IHC, X400). (c) Photomicrography of monophasic synovial sarcoma depicting the SMA negativity in tumour cells (IHC, X1000).

Lymphoma was diagnosed in 24 cases out of 54 cases based on CD45 positivity. Further, the diagnosis of HL was based on CD15 and CD30 markers. In addition, NHL was further categorised into T cell and B cell types based on positive reactivity with CD20 and CD3, respectively. Myogenin positivity was seen in 100% of

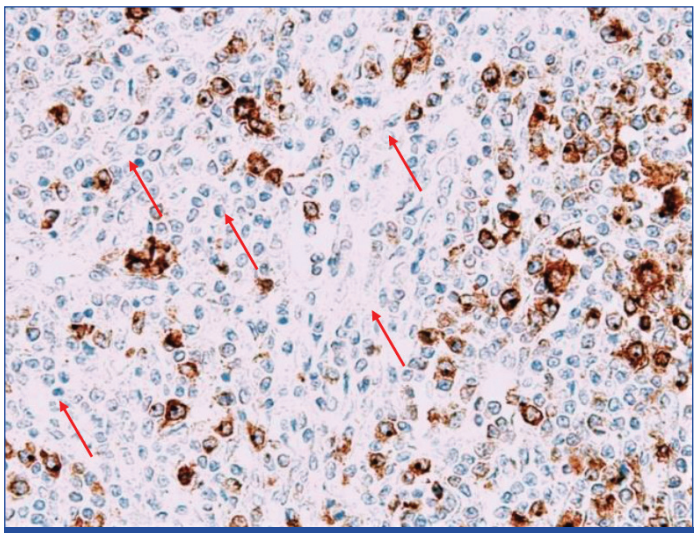


[Table/Fig-6]: Photomicrograph of Rhabdomyosarcoma. (a) Photomicrography of rhabdomyosarcoma (H&E, X1000) viewing heterogenous shapes of round to ovoid tumour cells with moderate eosinophilic cytoplasmic. (b) Photomicrograph of rhabdomyosarcoma showing positive nuclear staining for myogenin.

cases of Rhabdomyosarcoma. These cases showed heterogenous shapes of round to ovoid tumour cells with moderate eosinophilic cytoplasmic. The intercellular space showed a pale mucoid appearance [Table/Fig-6(b)]. SYP and chromogranin positivity was noted to diagnose Neuroblastoma [Table/Fig-4]. Both cases of LCH showed CD1a positivity Ewing's Sarcoma showed characteristic reactivity with CD99 on cell membranes [Table/Fig-7a,b]. Anaplastic large cell lymphoma with CD30 membranous positivity, immunohistochemical picture shown in [Table/Fig-8]. Compared with SYP, H&E was more sensitive, although less specific, in the identification of neuroblastoma.



[Table/Fig-7]: Photomicrograph of Ewing's Sarcoma (ES). (a) Photomicrograph of Ewing's Sarcoma showing round tumour cells (H&E, X400). Pseudorosette-like arrangement of the tumoural cells is shown but no fibrillary tangles are seen. (b) Photomicrograph of Ewing's Sarcoma showing strong CD99 membranous positivity (H&E, X400).



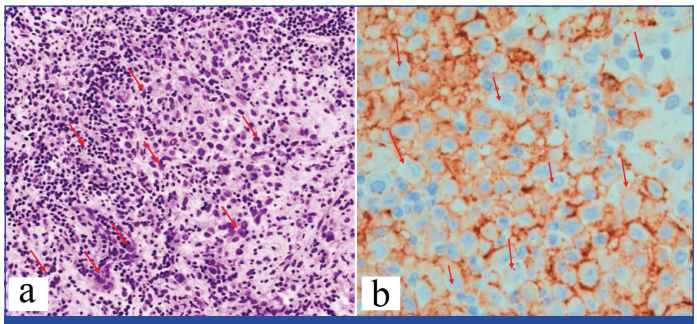
[Table/Fig-8]: Photomicrograph of anaplastic large cell lymphoma showing CD30 membranous positivity (IHC, X400).

Vimentin was found to be expressed in the cytoplasm of mangy tumour cells, according to the findings of the IHC staining study. According to this theory, patients with lymph node metastases (n=15) have considerably greater levels of vimentin protein expression than patients who do not contain metastases lymph nodes (n=24). IHC staining analysis revealed that vimentin overexpression was strongly linked to lymph node metastases in patients when taken as a whole [Table/Fig-5b]. SMA is a marker that can be used to identify smooth muscle, myofibroblastic, and associated cancers. However, because positive tumours can harbour a variety of other organisms, SMA positivity is insufficient in and of itself to diagnose smooth muscle differentiation [Table/Fig-5c].

Morphologically, RMS cells are of heterogeneous shapes ranging from undifferentiated and round cells to ovoid cells, microscopically, this tumour was composed of round to oval cells with a moderate amount of cytoplasm. In this view, nuclei were moderately pleomorphic with coarse chromatin and inconspicuous nucleoli [Table/Fig-6].

Strong CD99 membranous staining was the primary indicator in the cluster analysis for ES, and it was seen in 67% of these tumours. Membranous CD99 staining, in contrast to H&E, X400, demonstrated weak specificity for ES, as demonstrated by high staining of synovial sarcomas in 17% [Table/Fig-7].

Strong diffuse positivity for CD30, as is shown in anaplastic large cell lymphoma [Table/Fig-8] and moderate localised staining for EMA were realised during immunohistochemical analyses. Oval-shaped nuclei, grooves, and invaginations on H&E, X400 are distinguishable features with positive CD1a immunostains. From this, the results define that CD1a is most consistent with the diagnosis of LCH [Table/Fig-9a,b].



[Table/Fig-9]: Photomicrograph of Langerhances Cell Histiocytosis (LCH). (a) Photomicrograph of Langerhans Cell Histiocytosis (LCH), which shows oval cells with grooved nuclei and lymphocytes (H&E, X400). (b) Photomicrograph of Langerhans Cell Histiocytosis (LCH) showing CD1a membranous positivity (IHC, X1000).

DISCUSSION

Due to recent improvements in diagnostic and auxiliary techniques, SRBCT have proliferated exponentially and become increasingly complex. This type of neoplasm typically affects people of all ages and is distinguished by high cellularity, small cell diameters, sparse, and generally diffuse patterns of growth. These tumours exhibit malignant cells with basophilic staining nuclei and scanty cytoplasm; thereby giving it a bluish overall appearance on the slide; hence the more commonly known name "Small Round Blue Cell Tumour (SRBCT)" [11-14]. Historically, Ewing's sarcoma/PNET, neuroblastoma, lymphomas, and rhabdomyosarcoma have been the primary SRBCT group members. Diagnosing these tumours had been a major challenge in the past but with the advent of ancillary techniques and with the combination of topographic, morphologic and ultrastructural features, navigating these diagnostic dilemmas has become easier over the last two-three decades [14].

Since SRBCT differ biologically and genetically and have a wide range of histomorphological variations, it is crucial to distinguish them from other types of tumours. The histopathological examination has been the gold diagnostic in diagnosing tumours but the employment of a panel of antibodies has made it possible for pinpointing the diagnosis in even smudgy cases where histopathology alone cannot be enough for prognostication of the tumour. Selective use of a panel of IHC markers has made the diagnosticating even better. Immunohistochemical findings interpretation is highly specific, sensitive and requires a high level of competence because it frequently has a considerable impact on the final diagnosis. IHC should therefore always be used in conjunction with histological diagnosis, to strengthen the final analysis and avoid any possible diagnostic pitfall. Therefore, an appropriately chosen panel of antibodies is of paramount importance for arriving at an accurate diagnosis and ruling out the overlapping morphological features.

In the present study, 54 cases of SRBCT in patients under the age of 18 were found and examined. This stated that men predominated (74% of patients were men and 26% were women), and the majority of cases had occurred in the 10-≤15 year age range. The studies' sex distribution and male-to-female ratio were consistent with those of research by Ashraf MJ et.al, Bhagat VM et.al., D'cruz L et al., [11-14]. Additionally, there were 11 distinct types of small round cell tumours included in the study. The majority of these were NHL which contributed to 22.2% of all cases which was in concordance with the studies conducted by D'Cruz L et al., and Patel RG et al., [13,14]. Patel A et al., and Patel MM et al., the highest incidence of NHL was observed in malignant SRBCT [15,16]. They have been referred to as "Ewing's sarcoma-like" despite lacking the pathognomonic classical EWSR1-ETS translocation of ES because of partial morphologic overlap with ES and weak/patchy, the only constant immunological profile is CD99 expression. The others included Ewing's/PNET contributing to (18.5%) of total cases. Conrad P et al., showed 94.7% of cases [17]; and the findings of the investigation were corroborated by Patel MM et al., who demonstrated that CD99 was positively correlated in all cases of Ewing's sarcoma/PNET [16].

Immunohistochemically, strong membrane staining for CD99 is consistently seen in almost all cases of Ewing's sarcoma/Primitive Neuro-Ectodermal Tumours (PNET), although it is not very specific as it may also come positive in several other soft tissue sarcomas and lymphoblastic lymphomas. Although it is not very specific because it can also be positive in several other soft tissue sarcomas and lymphoblastic lymphomas. When ES/PNET is investigated immunohistochemically, CD99 staining strong membrane is frequently observed. In this study, nine cases of Rhabdomyosarcoma contributed to (16.6%) of the total cases which were in concordance with the studies conducted by D'Cruze L et al., Patel RG et al., and Konrad P et al., [13,14,17]. All of the studies showed 100% positivity for myogenin which corroborates with the present study findings.

The HL (22.2%), neuroblastoma (7.4%), monomorphic synovial sarcoma (3.7%), LCH (3.7%), olfactory neuroblastoma (1.9%), ganglioneuroblastoma (1.9%). In 2010, Martin AW, elucidated that NHL shows when CD45 is positive for CD3, it is highly selective for T cells and is negative for B cells, based on the eosin and haematoxylin stained sections from 12 biopsies from different organs, the initial differential diagnosis was Non-Hodgkins lymphoma [18]. After IHC, 12 cases of NHL were further categorised as Lymphoblastic Lymphoma (five cases), Burkitt's Lymphoma (two cases), ALCL (one case) and NHL unclassified (four cases). All 10 cases diagnosed to be ES/PNET showed positivity with CD99 which was in concordance with the findings of the study conducted by Folpe AL et al., [19]. All four cases of neuroblastoma showed positivity with chromogranin (100%) and NSE which was similar to the studies done by Terada T whose carcinoids displayed SYP positivity (85%) and chromogranin positivity (62%) [20]. In undifferentiated or poorly differentiated tumours, IHC may not be as helpful, but this study had no difficulty in diagnosing all 54 cases. [Table/Fig-10] depicts the immunochemistry study analysis from the last five years with the present study [21-24].

Jarwani PB et al., study was conducted in tertiary care hospitals and cases diagnosed as STTs from January 2018 to December 2020 [25]. Here, the Incidence of malignancy increased with age, malignant tumours being most common in the fifth decade. Adipocytic tumours (n=162, 63.8%) formed the major bulk of benign tumours. By examining the IHC expression of Ki-67 (MIB), neuron-specific enolase, SYP, and chromogranin in these tumours, Abdullah H et al., investigated the histological spectrum of NETs of the GIT [26]. The molecular markers CD99, CD20, CD45/LCA, CD3, SYP, EMA, Myogenin, chromogranin, desmin, and CK are used in the current investigation. SRBCT are therefore a subset of extremely aggressive malignant neoplasms.

References	Study area	Cases	Period	Results	Limitations/Future scope
Rajan A and Prema NS [21] 2019	Thiruvananthapuram	56 cases	January 2004 to December 2008	37 were Neuroblastomas (67%), 8 Ganglioneuroblastomas (15%) and 10 Ganglioneuromas (18%). Neuroblastoma received was differentiated-26 cases (70%).	The incidence of ganglioneuromas is higher
Tripathi PK et al., [22] 2020	Cuttack	63 patients	January 2013 to December 2017	One 14-month-old female infant was found to have PCC, there were 11 cases of adrenal tumours, eight of which were adrenal neuroblastomas, and there were two female children with cushing syndrome and virilisation at ages 3 and 7.	Needs a multidisciplinary approach to analysis
Murthy SS et al., [23] 2021	Hyderabad	81 patients	September 2009 to August 2019	In 89.83% of EWSR1 rearranged tumours, FISH revealed ESFT. 79.10% of the time, FISH confirmed the IHC diagnosis. FISH helped diagnose 1.49% of cancers that were CD99 negative.	CIC-DUX4 and BCOR studies have not been performed which would have completed the spectrum of round cell sarcomas
Bargunam P et al., [24] 2022	Shimoga	224 cases	From January 2007 till December 2019,	Benign myxoid lesions of neural origin with myxoid neurofibroma constituting 65 (29. 01%) cases, followed by schwannoma 38 (16.9%). Maximum benign lesions in the age group of 21-30, intermediate lesions (41-60) and malignant lesions >60 years.	Ancillary techniques for accurate diagnosis
Present study	Ludhiana	54 cases	August 2010 to July 2015	Small Round Blue Cell Tumours (SRBCT) analysis includes 12 cases of NHL, 12 cases of HL, 10 cases of Ewing's/PNETs, 9 cases of rhabdomyosarcoma, 4 cases of neuroblastoma, 2 cases each of LCH and synovial sarcoma, and 1 case each of olfactory neuroblastoma, sarcoma and ganglioneuroblastoma.	Few undifferentiated/poorly differentiated tumours

[Table/Fig-10]: Immunohistochemistry (IHC) spectrum study of tumour cells comparison analysis with present work [21-24].

Limitation(s)

Firstly, the limited size of the patient population, secondly non availability of other modalities and markers for being an adjunct to the results were the limitations. Moreover, there were few undifferentiated/poorly differentiated tumours for which due to financial restraint and financial burden on the patient party, the authors could not apply the whole panel of IHC markers and could not arrive at a final diagnosis. Such cases had to be excluded from the study.

CONCLUSION(S)

The SRBCT are a heterogeneous group of malignant neoplasms. IHC presents a rapid and cost-effective ancillary tool that can provide a clear-cut distinction among these various tumour types. A diverse category of malignant neoplasms is known as SRBCT. IHC is a fast and low-cost supplementary tool that can distinguish between these various cancer forms. Monoclonal antibodies CD45/LCA, CD20, CD3, CD99, Myogenin, desmin, EMA, CK, SYP, chromogranin, S100, CD34, ALK, MIC-2, CD1a BCL-2, BCL-6, and CD10 were applied on FFPE tissue sections. Its primary objective is to group patients according to their histomorphological patterns to guarantee that they receive an appropriate and targeted treatment that is well-tailored to their needs. It also aims to detect tumours that are more likely to result in catastrophic consequences and recurrence thereby ultimately improving the treatment outcome and final prognostication. IHC should be carried out to a high degree for the results to be beneficial and repeatable because it can provide such crucial information.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, Adesh Institute of Medical Sciences and Research, Bathinda, Punjab, India.
2. Associate Professor, Department of Pathology, PDCC Renal and Transplant Pathology, Adesh Institute of Medical Sciences and Research, Bathinda, Punjab, India.
3. Assistant Professor, Department of Pathology, Adesh Institute of Medical Sciences and Research, Bathinda, Punjab, India.
4. Assistant Professor, Department of Paediatric Surgery, Adesh Institute of Medical Sciences and Research, Bathinda, Punjab, India.
5. Professor, Department of Pathology, Dayanand Medical College and Hospital, Ludhiana, Punjab, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sumit Dhuria,
Assistant Professor, Department of Paediatric Surgery, Adesh Institute of Medical Sciences and Research, Bathinda-151109, Punjab, India.
E-mail: sumitdhuria04@outlook.com; sumitdhuria1984@gmail.com

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