Evaluation of Pediatric Common Solid Small Round Cell Tumors: An Immunohistochemical Study

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Abstract

Background: Pediatric small round cell tumors (SRCTs) are diagnostically challenging lesions due to their primitive character. With the rising incidence and having better treatment outcome as compared to the past, the categorization of SRCTs into definitive histological types is extremely important as individual tumor differs therapeutically and has separate prognostic significance. Immunohistochemistry (IHC) can play an important role here.

Objective: The present study was designed to evaluate the role of immunohistochemistry (IHC) in the differential diagnoses of pediatric SRCTs.

Results: In this study, various histomorphological types of pediatric SRCTs were identified in about 97% of cases with the aid of immunohistochemical stains. However, about 3% of cases remain unclassified even after immunohistochemical tests. The different morphological patterns were as follows; 24.4% rhabdomyosarcoma, 22.2% lymphoblastic lymphoma, 22.2% neuroblastoma, 22.2% soft tissue Ewing sarcoma, 4.44% Wilms Tumor, and 1.48% poorly differentiated synovial sarcoma.

Conclusion: Immunohistochemistry plays an important role as is evident from the present study and supported by many previous studies in categorizing undifferentiated or poorly differentiated small round cell tumors of childhood.

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Keywords: Small round cell tumors (SRCTs), Immunohistochemistry (IHC)

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Introduction

Childhood cancers include a spectrum of different malignancies that constitute both hematological malignancy and solid neoplasms (benign and malignant).[1] The proportion of childhood cancer burden in Bangladesh is high as evident from different previous studies.[2] Among the solid malignant tumors, a diverse group of highly malignant tumors with basic primitive morphological features of small round cells is called "Small Round Cell Tumors (SRCTs)".[3] These tumors typically present as a vast sea of dark-blue nuclei on routine hematoxylin and eosin stains.[4] The common SRCTs include Neuroblastoma (NB), Lymphoblastic lymphoma (LBL), Rhabdomyosarcoma (RMS), Ewing sarcoma (EWS), Wilms tumor (WT), Hepatoblastoma (HB), Retinoblastoma (RB), Poorly differentiated synovial sarcoma (PDSS) and Desmoplastic small round cell tumors (DSRCT).[5] Differential diagnosis of small round cell tumors is particularly difficult in routine histopathology due to their undifferentiated or primitive character. Accurate categorization of SRCTs has become increasingly important as modern therapies are not only disease-specific but also tailored according to patient risk. Over the years, the survival rate in pediatric SRCTs has drastically improved mostly due to the combined modality of management. IHC has been a prevalent and convenient method of surgical pathology to categorize many histologically non-conclusive neoplasms.[6] However, sometimes overlapping IHC features cause a diagnostic dilemma. For establishing the correct diagnosis, an appropriate selection of IHC stains and an accurate interpretation of staining patterns are necessary.[3,6] With the current increasing demand, the present study is designed to evaluate the role of IHC in diagnosing pediatric SRCTs.

Methods

This cross-sectional observational study was carried out in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2020 to February 2022. After getting permission from the Institutional review board (IRB), hundred and thirty-five histologically diagnosed cases of SRCTs were included. All hematoxylin and eosin (H&E) stained slides and their corresponding formalin-fixedparaffin-embedded (FFPE) tissue blocks were collected from the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU). Relevant information and data were collected from hospital records on a prescribed proforma. From FFPE tissue blocks, 5-micrometer thick sections were cut, deparaffinized in hot air woven at 58°C for one hour, dewaxed in xylene, and rehydrated through a graded series of isopropyl alcohol. Antigen retrieval was done by treating the slides with Dako Target Retrieval solutions, each with a specific p^H for each antibody. Solutions were taken in a Coplin jar and pre-heated in the water bath at 65ºC. Then slides were placed in the antigen retrieval solution and heated in the water bath at 95-99°C for 30-40 minutes. Then the sections were stained subsequently with a panel of antibodies selected based on histological diagnosis as per the need of individual cases. IHC staining procedures were done according to the standard protocol followed by the Pathology Department of BSMMU using DAKO EnVision TM FLEX+, High pH (code: K8012) detection system. This system detects primary Mouse and Rabbit monoclonal antibodies and the reaction was visualized by EnVision TM FLEX DAB+ Chromogen. Immunohistochemical staining for each antibody was interpreted qualitatively as either positive (+), or negative (-).Positive control was examined for the presence of colored end product at the site of the target

antigen (DAB chromogen – brown end product). The presence of the brown color in the cytoplasm, nucleus, or cell membrane (based on antigen site) was interpreted as a positive staining result, indicating the proper performance of the reagents. Then, the test specimens stained with primary antibodies were examined. The absence of a colored end product was interpreted as a negative result.

Results

In this present study immunohistochemical evaluation was done in 135 cases of SRCTs. The retroperitoneum was the most common site of origin (62.2%). The age of the patients ranged from nine months to 17 years with a mean of 8.9 years. The male was predominant and to female ratio was 1.65:1. After a thorough immunohistochemical evaluation, 131 (97%) cases were conclusively categorized into six variants including 33 RMS (24.44%), 30 LBL (22.22%), 30 NB (22.22%), 30 soft tissue EWS (22.22%), six WT (4.44%) and two cases of PDSS (1.48%) (Fig-1). Four cases remained unsolved.

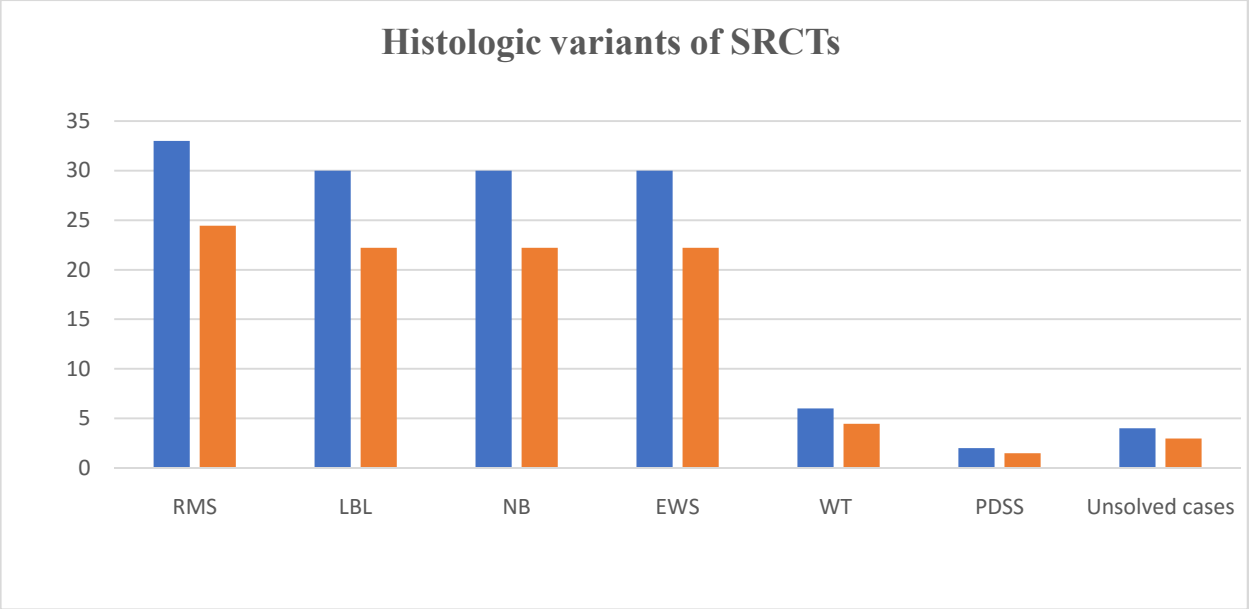


Figure 1. Morphological variants of SRCTs after IHC

All 33 (24.44%) cases of RMS showed positive cytoplasmic reactivity for desmin, whereas, myogenin was positive in 29 (21.48%) cases of RMS (Fig- 2). Itwas observed that the myogenin expression pattern for embryonal rhabdomyosarcoma was found patchy nuclear whereas it showed diffuse nuclear positivity in alveolar rhabdomyosarcoma (Fig- 3). Myogenin was found to be negative in all other cases of SRCTs. Desmin was however positive for one case of PDSS and negative for all other SRCTs.

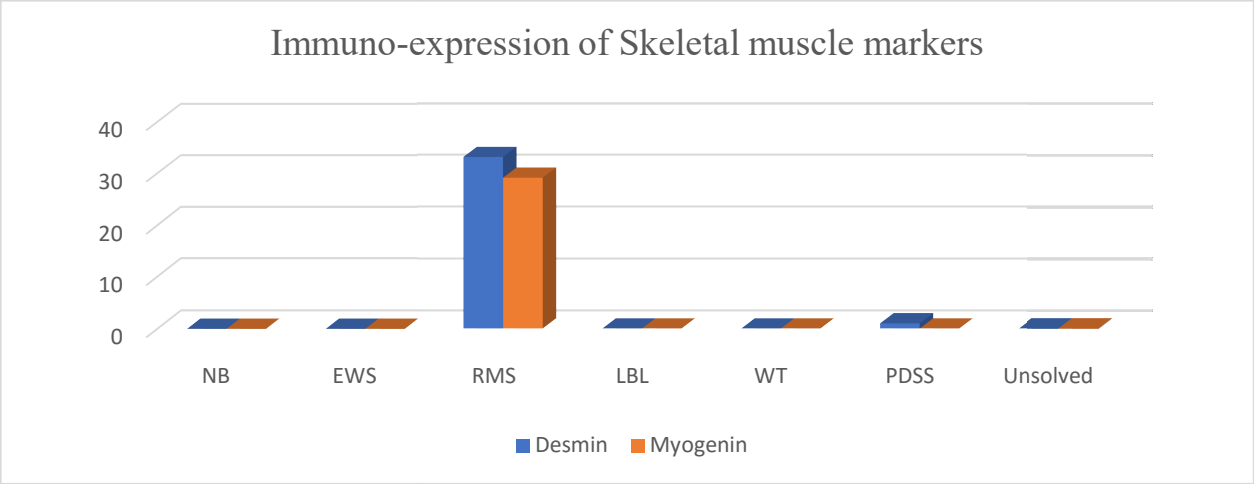


Figure 2. Expression of Skeletal muscle markers (Desmin and Myogenin) in SRCTs

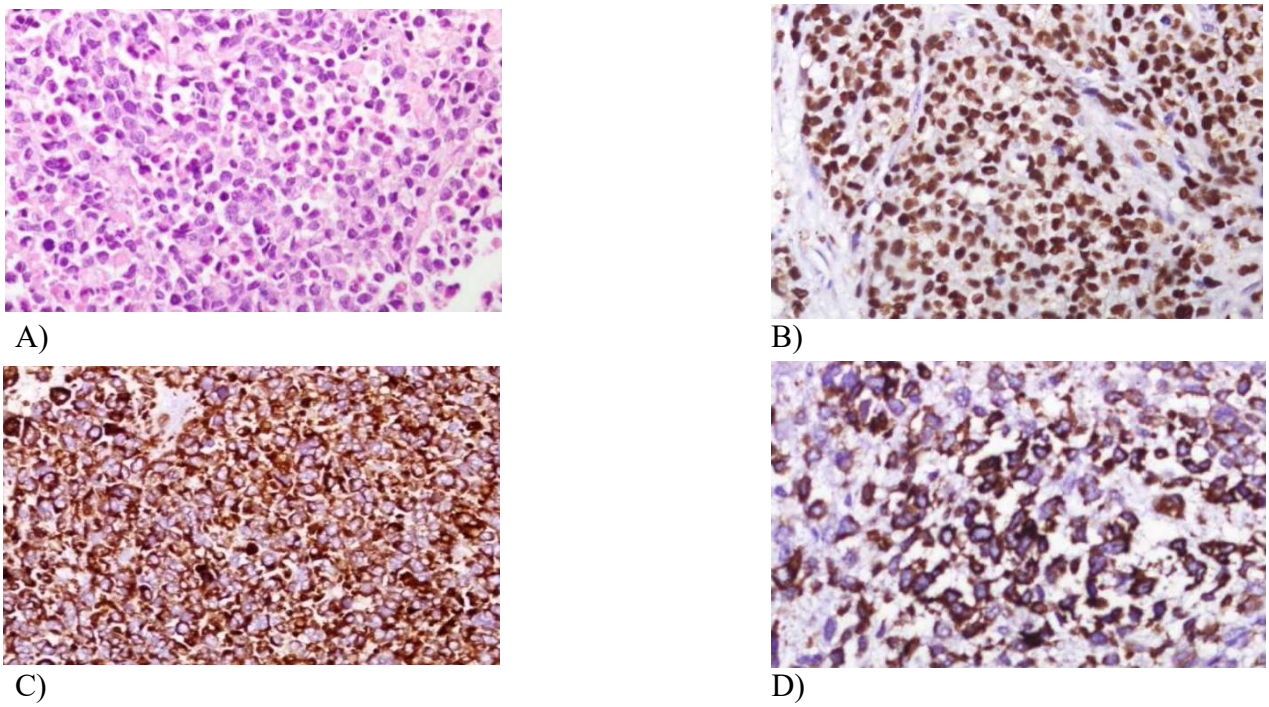


Figure 3. Photomicrograph of a case of Alveolar RMS (400X). A, H&E. B, Myogenin. C, Desmin. D, NSE.

Neuroendocrine markers such as neuron-specific enolase (NSE), synaptophysin, CD56 and chromogranin were used in the present study to delineate various types of SRCTs (Fig-4). These markers were collectively expressed in all 30 cases (22.22%) cases of NB (Fig-4). Eight cases of RMS and 27 cases of soft tissue EWS also showed positive reactions for different neuroendocrine markers (NSE and synaptophysin). A single case of PDSS was positive for CD56. Chromogranin was highly specific for NB (Fig- 5).

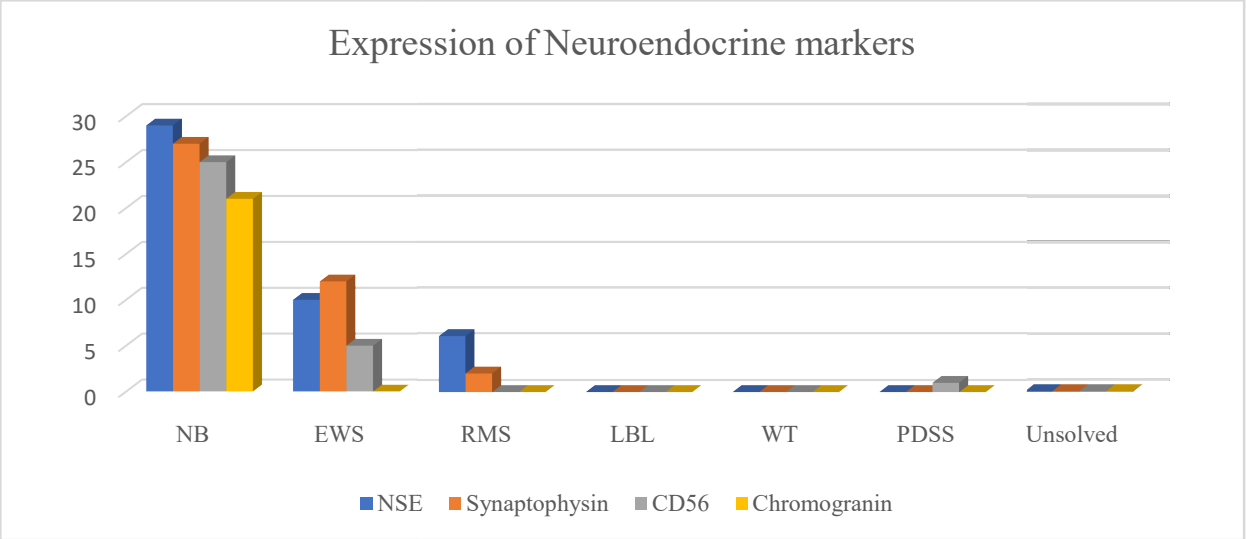


Figure 4. Expression of Neuroendocrine markers in SRCTs

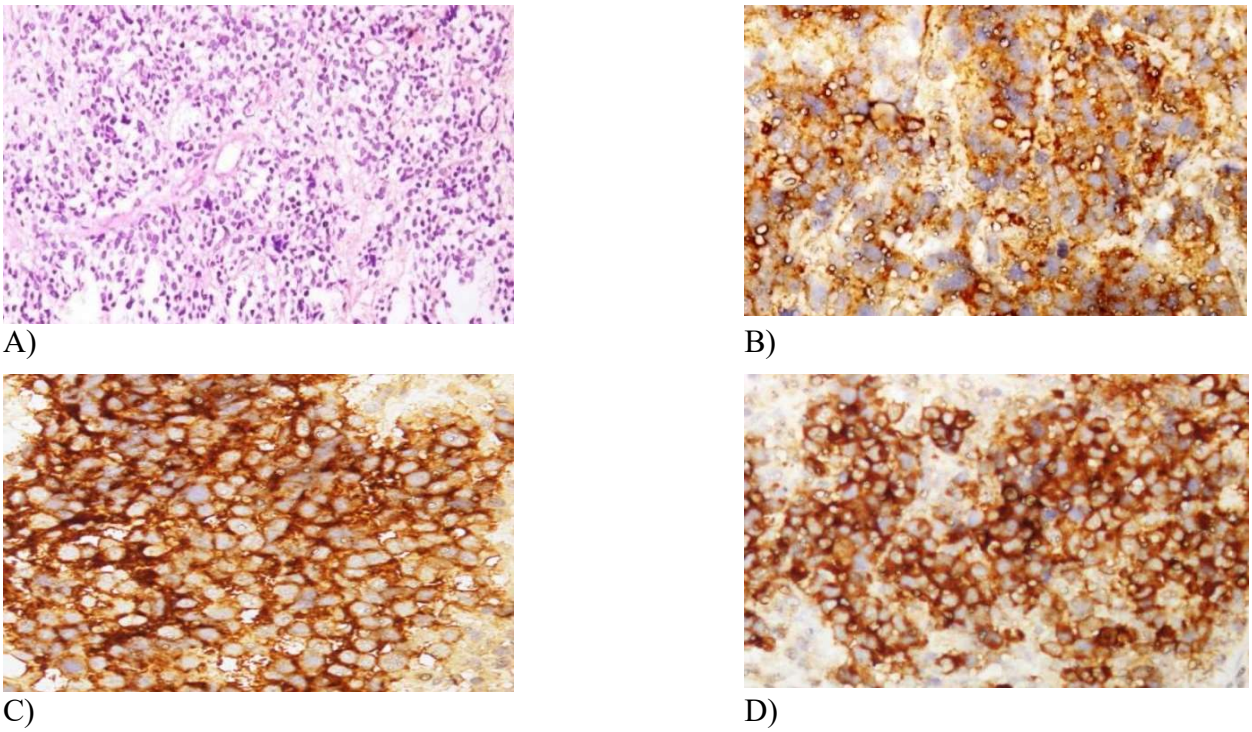


Figure 5. Photomicrograph of a case of neuroblastoma (400X). A, H&E. B, Chromogranin. C, Synaptophysin. D, CD56.

Two blastic markers CD99 and TdT were used in this study. All 30 cases of LBL (both B and T cell types) show positive nuclear reactions for TdT. Twenty-eight cases show CD99 and TdT co-expression. LCA (CD45) was used only in 19 cases to distinguish LBL from other SRCTs. All the 19 cases showed positive reactions for LCA. All other cases of SRCTs were negative for TdT and LCA (Fig- 6). CD99 also shows the characteristic diffuse strong membranous reaction in all the cases of soft tissue EWS (Fig-8).

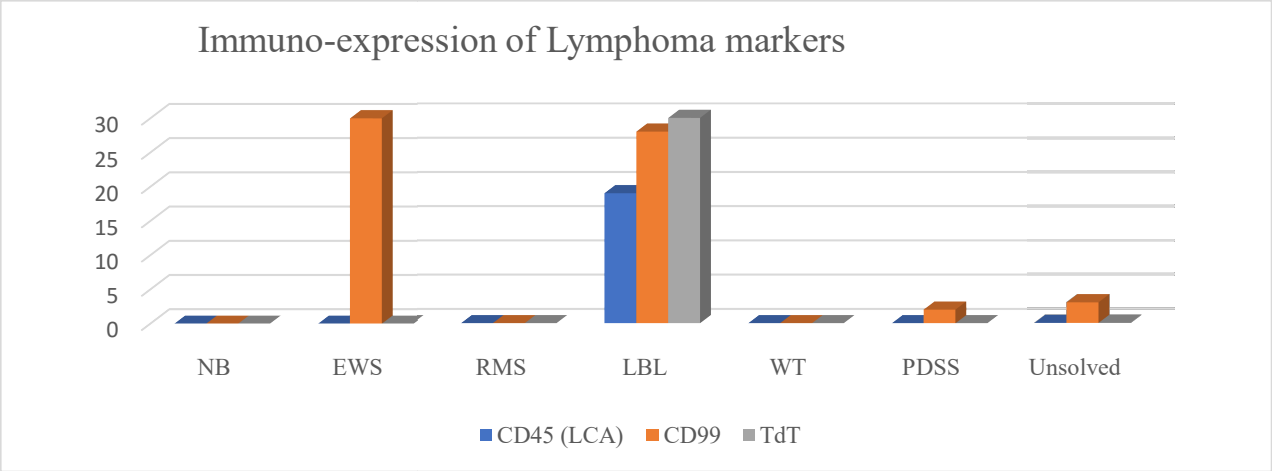
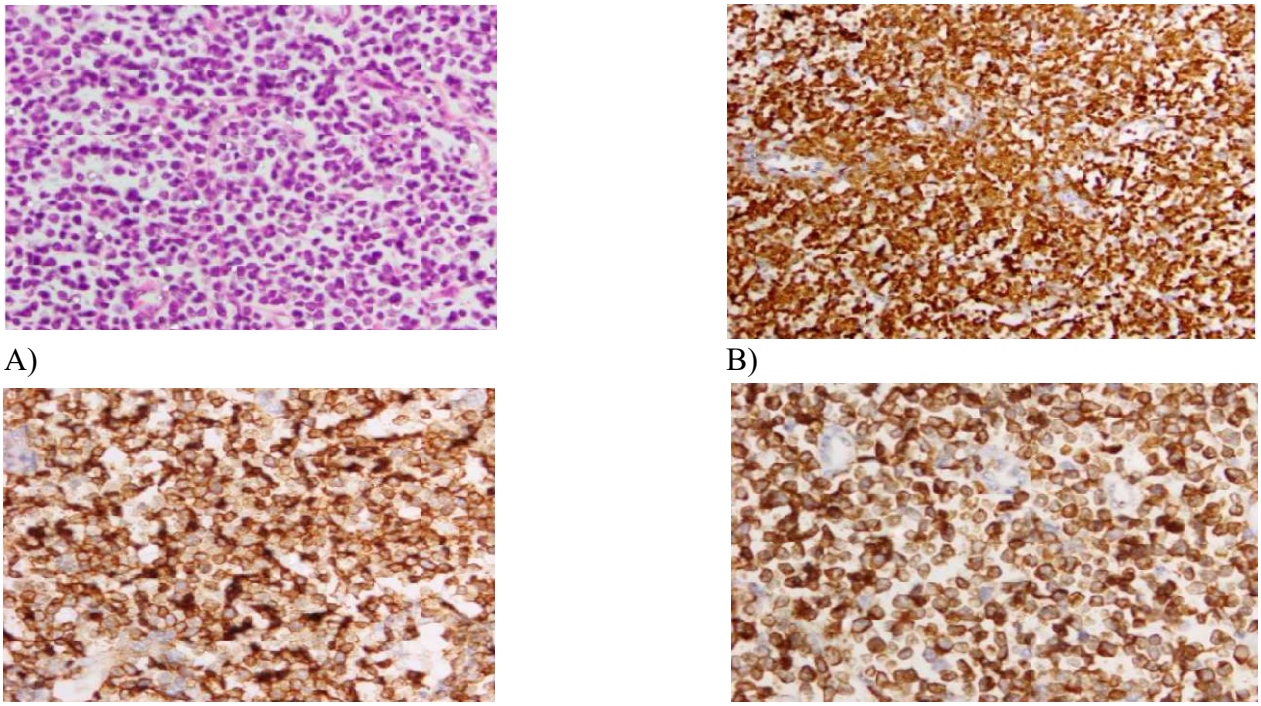


Figure 6. Expression of lymphoma markers in SRCTs

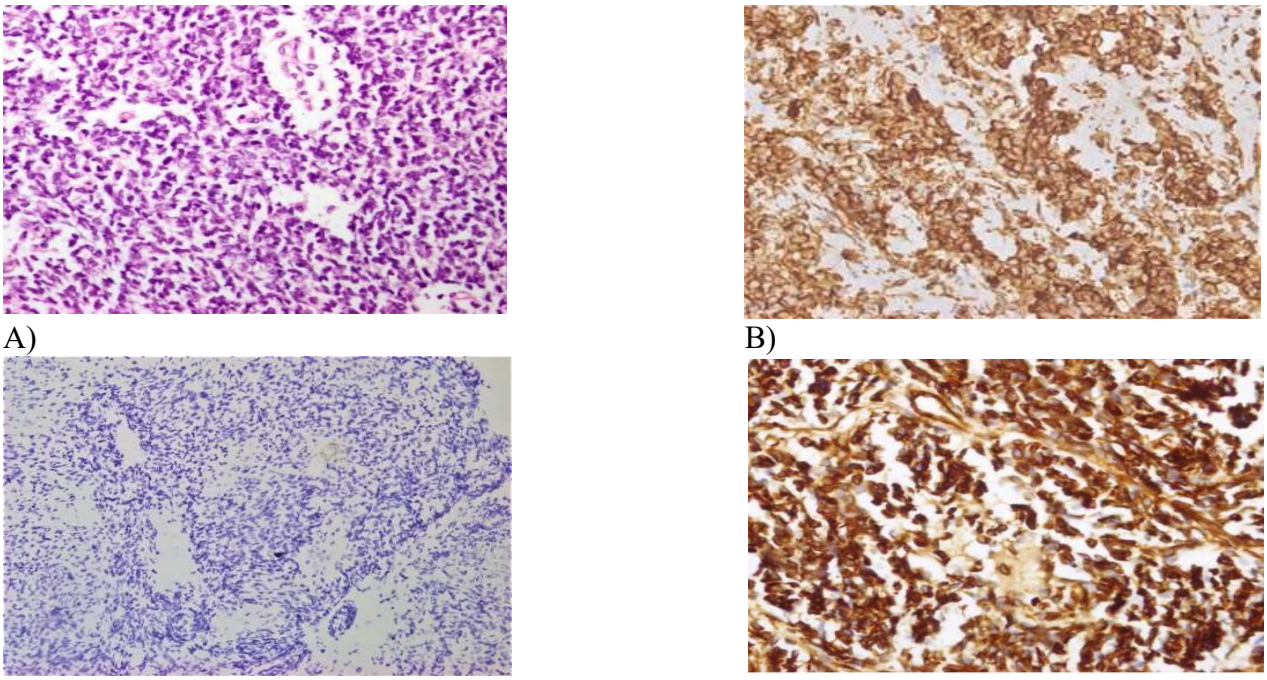
Two cases of PDSS and three unsolved cases also show focal positive reactions for CD99. CD20, PAX5, CD79α, CD3, CD5, CD10, and Ki 67 were used for the further categorization of LBL into B and T cell types (Fig- 7). Twenty-four cases were T- Lymphoblastic Lymphoma (T-LBL) and six were B-Lymphoblastic Lymphoma (B-LBL).



A) B)

C) D)

Figure 7. Photomicrograph of a case of T-LBL (400X). A, H& E. B, TdT. C, CD3. D, CD99.



A) B) C) D)

Figure 8. Photomicrograph of a case of EWS (400X). A, H&E. B, CD99. C, TdT. D, Vimentin.

Six cases of WT composed of blastemal components only were included. All of which were received as retroperitoneal mass. All of these showed strong nuclear reactivity for WT-1 (Fig-9). WT-1 was also used in 25 cases of RMS, all of which showed strong cytoplasmic positivity.

A) B)

Figure 9. Photomicrograph of a case of Wilms Tumor (blastemal only)(400X). A, H&E. B, WT-1.

Vimentin was performed in 71 cases of SRCTs, of these 33 RMS, 22 EWS, two PDSS, and four US cases showed positive cytoplasmic reaction. Vimentin was also used in 10 cases of NB, all of which showed a negative reaction. 39 out of 135 cases of SRCTs were subjected to Pancytokeratin (AE-1/AE-3) immunostaining; onlytwo cases of PDSS were found focallypositive. EMA, BCL-2, and S-100 were used as secondary markers in all two cases of PDSS and four US cases. All three markers were positive in both cases of PDSS. S-100 and BCL-2 were positive in one unsolved case. EMA showed a negative reaction for all four cases of US. Glypican 3 was used in a single hepatic

metastatic case of neuroblastoma to distinguish it from small-cell undifferentiated hepatoblastoma. It was found to be negative.

Discussion

In this cross-sectional observational study, a total of 135 cases of SRCTs (any site of origin and any type of surgical procedure) were included. The majority of cases (71%) occurred below 10 years male and the frequency gradually decreased with increasing age. Most of the samples were received as retroperitoneal mass (62.2%). Several previous studies showed that SRCTs predominantly occurred in males within 10 years of age.[7,8] After IHC six morphological variants were observed among 131 conclusive cases. RMS was the most common subtype (24.44%). Some recent studies showed RMS was the most common pediatric soft tissue SRCT.[9,10] However, in other studies, it was observed that LBL was the most frequently diagnosed tumor among children.[11] This may be because of a small sample size that is not representative of the population and geographical variation of pediatric tumors. It was noticed from this study that approximately 97% of the SRCTs were accurately diagnosed with the help of immunohistochemistry and only 03% were inconclusive even after immunohistochemical tests. Similar findings were reported by some previous studies.[11,12]

Among the 33 cases of RMS, 29 (88%) cases were of embryonal rhabdomyosarcoma (ERMS) (91%) and four cases were alveolar rhabdomyosarcoma (ARMS) (12%). Several previous studies reported that ERMS is more common than ARMS in children. [13] In the present study, all 33 cases of RMS were stained with desmin, myogenin, and vimentin. 29 (88%) cases showed co-expression of desmin, myogenin, and vimentin. Desmin and vimentin were co-expressed in all 33 cases of RMS. Only four cases of ERMS showed a negative reaction to myogenin. Myogenin is a specific less sensitive marker for RMS and a small percentage (>1%) of nuclear positivity is considered positive.[14] Desmin is more sensitive but less specific as it is often found to be positive in other small round cell tumors.[10] In the present study, desmin is also positive in one case of PDSS. Some other aberrantly expressed immunohistochemical markers in RMS include NSE (6/33), and synaptophysin (2/33). These neuroendocrine markers were found focally positive and did not alter the diagnosis. Some immunomarkers used to distinguish between RMS and other SRCTs, were negatively stained including CD45, CD99, TdT, CD56 and chromogranin.

LBL, NB, and EWS showed equal frequency (22.2%) and are the second most common SRCTs noted in the present study. Most of the LBL occurred within the lymph node (19/30) and others (11/30) cases were reported within the soft tissues.It was noted T-cell lymphoblastic lymphoma (T-LBL) (80%) was morecommon than B-cell lymphoblastic lymphoma (B-LBL) (20%). This finding is similar to some recent studies.[6] 28 cases showed CD99 and TdT co-expression. Two cases of T-LBL where TdT was negative, showed positive reactions for CD99 and negative reactions for vimentin. T-LBL showed positive reactions for CD3 and CD5; whereas, CD20, CD79α, and PAX5 were positive in B-LBL. Ki-67 showed strong nuclear reactivity in more than 85% of tumor cells in each case of LBL. However, two cases of T-LBL showed positive reactions for CD79α. A recent study reported that CD79α was positive in 10% of cases of T-LBL.[15]

In the present study, retroperitoneal soft tissue was the most common site for neuroblastoma. Neuroblasts are reactive for a variety of markers. Some of these markers are highly sensitive with lack specificity, such as NSE, synaptophysin, and CD56. On the other hand,

the more specific markers such as, chromogranin is less sensitive.[3] It was observed that NSE being the most sensitive marker was found positive in 97% of neuroblastoma cases as well as other small round blue cell tumors (10 cases of EWS and 6 cases of RMS), whereas chromogranin being the most specific marker was found to be positive in 21 cases(70%) of neuroblastoma and negative in all other SRCTs. Similarly, CD56 and synaptophysin showed positive reactions in other SRCTs (EWS and RMS). With this observation, it can be deduced that one sensitive and one specific marker is sufficient to reliably diagnose neuroblastoma. CD45, CD99, and myogenin were used to differentiate neuroblastoma from other SRCTs in this study, which were found non-reactive.[3] Two cases of metastatic neuroblastomas; one was in the lymph node and one was noted in the liver. In the case of metastasis to the lymph node, CD45 was negative, and synaptophysin and NSE were positive. Metastasis to the liver, the tumor was indistinguishable from small cell undifferentiated hepatoblastoma, so glypican 3 was used and found to be negative. CD56 and NSE were positive. A recent study mentioned that 50 to 60% of neuroblastoma display metastasis at the time of diagnosis; the most common sites were regional lymph nodes, bone, and liver.[16]All 30 cases of EWS showed diffuse strong membranous positivity for CD99. Vimentin also showed strong cytoplasmic positivity in all the cases of EWS. NSE, synaptophysin, and CD56 showed positive reactions in some cases whereas, TdT was negative in 100% of cases of EWS.WT-1 showed a positive nuclear reaction in all six cases of Wilms Tumors. 25 cases of ERMS showed positive cytoplasmic reaction for WT-1. Wilms Tumors were negative for CD99, TdT, LCA, desmin, myogenin, and neuroendocrine markers. Two PDSS cases were diagnosed in this study, originating as soft tissue mass in the upper extremity. The initial IHC panel showed a positive reaction for vimentin, CD99 (focal), and pancytokeratin. Myogenin and LCA were negative. For further evaluation EMA, BCL-2, desmin, CD56, and S-100 were used. EMA, BCL-2, and S-100 were positive in both cases whereas, CD56 and desmin were focally positive in one case. Four cases remain unsolved even after the use of a large number of antibodies. The initial pane of antibodies includes LCA, pancytokeratin, vimentin, CD56, CD99, myogenin, and desmin. Out of these seven markers, vimentin showed diffuse strong cytoplasmic positivity in all four cases whereas, three cases showed focal positivity for CD99. Rest markers were negative. The second panel of immunostains was used, including EMA, BCL2, S-100, and TdT. EMA and TdT were negative in all four cases. BCL-2 and S-100 were positive in one case which was negative for CD99.Therefore, further investigations were needed to categorize this tumor.

Conclusion

From this study, it can be concluded that it is crucial to use a panel of antibodies, including the combined use of specific markers and sensitive markers to correctly diagnose a case of small round cell tumor. It is also evident from the present study that some cases remain unclassified after IHC, thereby requiring the need for further evaluations including, cytogenetic, molecular studies, and electron microscopy. Knowledge about the utilities and pitfalls of each immuno-marker is essential for avoiding potential diagnostic errors because a tissue-specific marker is exceptionally rare. However, IHC has greatly improved the ability to classify many histologically non-conclusive SRCTs in the pediatric population.

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