Evaluation of Cyclin D1 Expression in Paediatric Common Solid Small Round Cell Tumours

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Abstract

Background: Small round blue cell tumors (SRBCTs) in children are a heterogeneous group of neoplasms. Their diagnosis is very difficult due to their primitive character. Although the advent of immunohistochemistry has improved the quality of diagnosis, some cases require molecular analysis. However, the application of molecular tests is limited due to the lack of resources. Nuclear expression of Cyclin D1 can be used as a diagnostic adjunct to conventional markers in diagnosing small round cell tumors, especially when the diagnosis becomes difficult even following the application of conventional markers.

Objectives: This study aimed to evaluate the immunoreactivity of Cyclin D1 in the common solid small round blue cell tumors found in children below 15 years of age.

Methods: In this descriptive cross-sectional study, 64 confirmed (immunohistochemically and/or morphologically) cases of SRBCTs including, Ewing sarcoma (31.25%), neuroblastoma (23.44%), lymphoblastic lymphoma (21.87%), rhabdomyosarcoma (15.62%) and Wilms tumor (7.81%) in children under 15 years were selected as samples by inclusion and exclusion criteria. Finally, the immunoreactivity of Cyclin D1 in each case was assessed on the basis of staining pattern and staining intensity.

Result: All cases of Ewing sarcoma and neuroblastoma exhibited nuclear expression for cyclin D1. Seventy percent (14/20) of cases of Ewing sarcoma and 66.67% (10/15) of neuroblastoma cases showed diffuse nuclear expression. Seventy percent (14/20) of cases of Ewing sarcoma and 93.33% (14/15) of neuroblastoma cases showed strong staining intensity (3+). In contrast, this marker showed a negative reaction in rhabdomyosarcoma and lymphoblastic lymphoma, while it was focally positive with moderate intensity in the blastemal component of 40% of cases of Wilms tumor.

Conclusion: The present study suggests that cyclin D1 can be exploitable as a diagnostic adjunct to conventional markers in confirming the diagnosis of Ewing sarcoma or Neuroblastoma.

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Keywords: Small round blue cell tumors (SRBCTs), Cyclin D1

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Introduction

Childhood malignancies (< 15 years) constitute only a small fraction of the global cancer burden.[1] Most childhood cancer (defined here as cancer in children aged 0-14 years) occurs in developing countries where nearly 90% of the world's children live and have access to relatively poor health facilities.[2] In Bangladesh, children less than 15 years constitute about 35% of the total population and the proportion of childhood cancer burden in Bangladesh is high.[3] Among the childhood malignancies, a diverse group of highly malignant tumors, showing basic morphological features of small round blue cells exists. Their primitive character makes small round blue cell tumors (SRBCTs) diagnostically challenging.[4] The most common tumors include rhabdomyosarcoma (RMS), Ewing sarcoma (EWS), neuroblastoma (NB), lymphoblastic lymphoma (LBL), and Wilms tumor (WT) (the blastemal component).[5] The application of IHC techniques along with routine light microscopic histology coupled with clinical and radiological features can diagnose and classify accurately more than 90% of cases of SRBCTs. However, some cases require molecular tests to confirm the diagnosis but the application of molecular tests in resource-poor settings like ours, its use is limited.[3,6]

Amplification or over-expression of cyclin D1 plays a vital role in developing various human cancers including breast cancer, colon cancer, melanoma, prostate cancer, and lymphoma for bypassing the G1–S phase.[7] Immunohistochemical expression of Cyclin D1 has not been evaluated systematically in solid SRBCTs of children and adolescents. In this regard, an immunohistochemical study performed on Ewing sarcoma found expression of Cyclin D1 in 42% of cases without significant correlation with survival.[8] Another immunohistochemical study reported Cyclin D1 over-expression in peripheral neuroblastic tumors.[9]In several emerging studies, cyclin D1 is the primary therapeutic target to control neoplastic cell proliferation.[10]

With the current increasing demand, the present study is designed to evaluate the potential usefulness of Cyclin D1 as an immunomarker in the differential diagnosis of common solid tumors exhibiting small round blue cell morphology in children below 15 years. Moreover, understanding the immunoreactivity of Cyclin D1 in solid small round blue cell tumors might offer new therapeutic options for clinicians.

Methods

Sixty-four confirmed cases of solid small round blue cell tumors were selected as samples for this study. Diagnoses are confirmed by well-established histopathological features and by immunohistochemistry (IHC) in related cases. From corresponding formalin-fixed paraffin-embedded (FFPE) tissue blocks, five micrometer thick sections were cut from each tissue block, and then the sections were deparaffinized in a hot air oven at 58° C for one hour and dewaxed in xylene. Then the sections were rehydrated through a graded series of alcohol. The sections were treated with the Dako Target Retrieval solution (pH 9) for antigen retrieval. Solutions were taken in a Coplin jar and pre-heated in the water bath at 65^0c. Then slides were placed in this solution and heated in the water bath at 95-99^0 c for 30-40 minutes. Then the sections were incubated in anti-cyclin D1 (EP 12, DAKO) (Ready to use rabbit antibody). IHC staining was done according to the standard protocol followed by the IHC laboratory of BSMMU using the DAKO EnVision™ FLEX+, High pH (code: K8012) detection system. In this system, the reaction is visualized by EnVision™ FLEX DAB+Chromogen (brown). The immunoreactivity of Cyclin D1was assessed by semi-quantitative optical

analysis according to a four-tiered system (< 1% positive cells, negative staining; 1% - 10 % positive cells, focal staining; 11% - 50% positive cells, heterogeneous staining; > 50% positive cells, diffuse staining) indicating the patterns of staining. Staining intensity was graded as strong or 3+ (nuclear intensity similar to that of the epithelial lining of tonsil control), moderate or 2+ (definite nuclear staining weaker than 3+ but easily identifiable at × 40 magnification), and weak or 1+ (nuclear staining identifiable only at high power magnification).[11,12]

Results

In this cross-sectional observational study, 64 confirmed cases of SRBCTs were included in this study. Among them the predominant population was male, and the male-to-female ratio was 1.67:1. Overall, 69 % of study subjects were within 10 years of age.

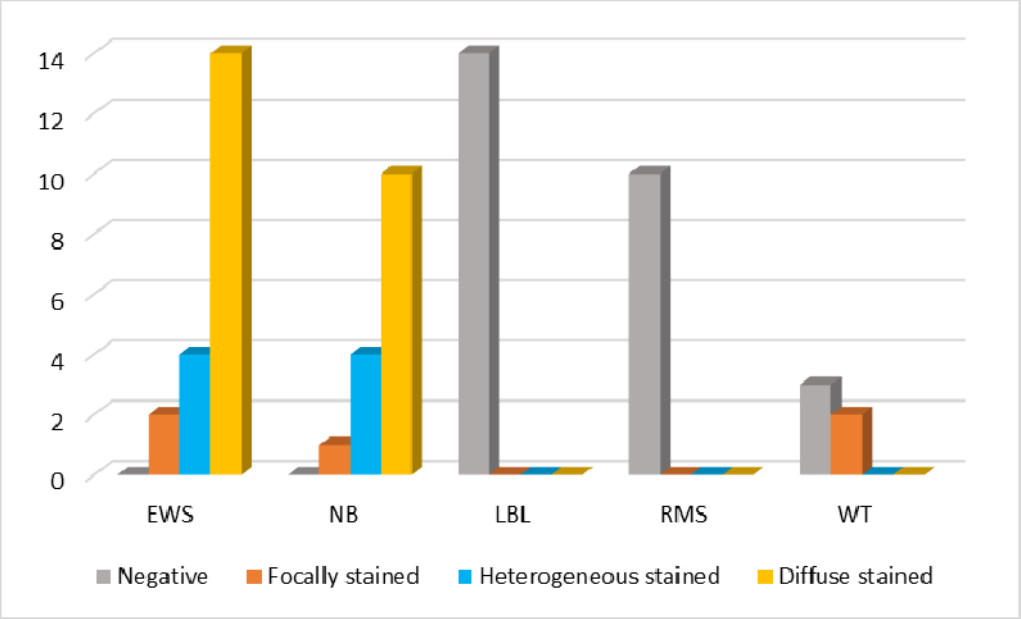

Figure 1. Multiple Bar diagram showing immunoreactivity of Cyclin D1 in SRBCTs

As far as Cyclin D1 immunohistochemical expression is concerned, 14 (70%) cases of EWS showed diffuse expression of Cyclin D1, four (20%) cases were heterogeneously stained and two (10%) cases were focally stained. In NBs, 10 cases (66.67%) were diffusely stained, four (26.66%) cases were moderately stained and the remaining one (6.67%) case was focally stained. The blastemal component of two (40%) WTs was focally stained. Fourteen (100%) cases of LBL, 10 (100%) cases of RMS, and three (60%) cases of WT showed negative immuno-expression for Cyclin D1 (fig-1). Strong staining intensity for Cyclin D1 was found in 14 (70%) cases of EWS, and 14 (93.33%) cases of NB. Six (30%) cases of EWS, the remaining one (6.67%) case of NB, and two (40%) positive cases of WT (in blastemal component) showed moderate staining intensity for Cyclin D1 (fig-2).

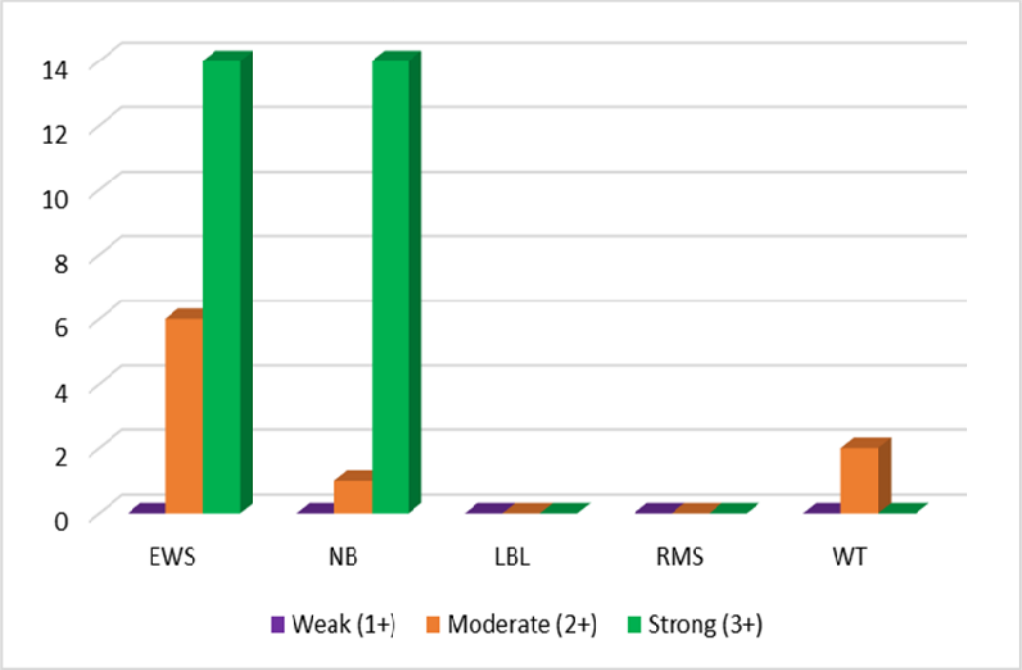

Figure 2. Multiple Bar diagram showing staining intensity of Cyclin D1 in SRBCTs

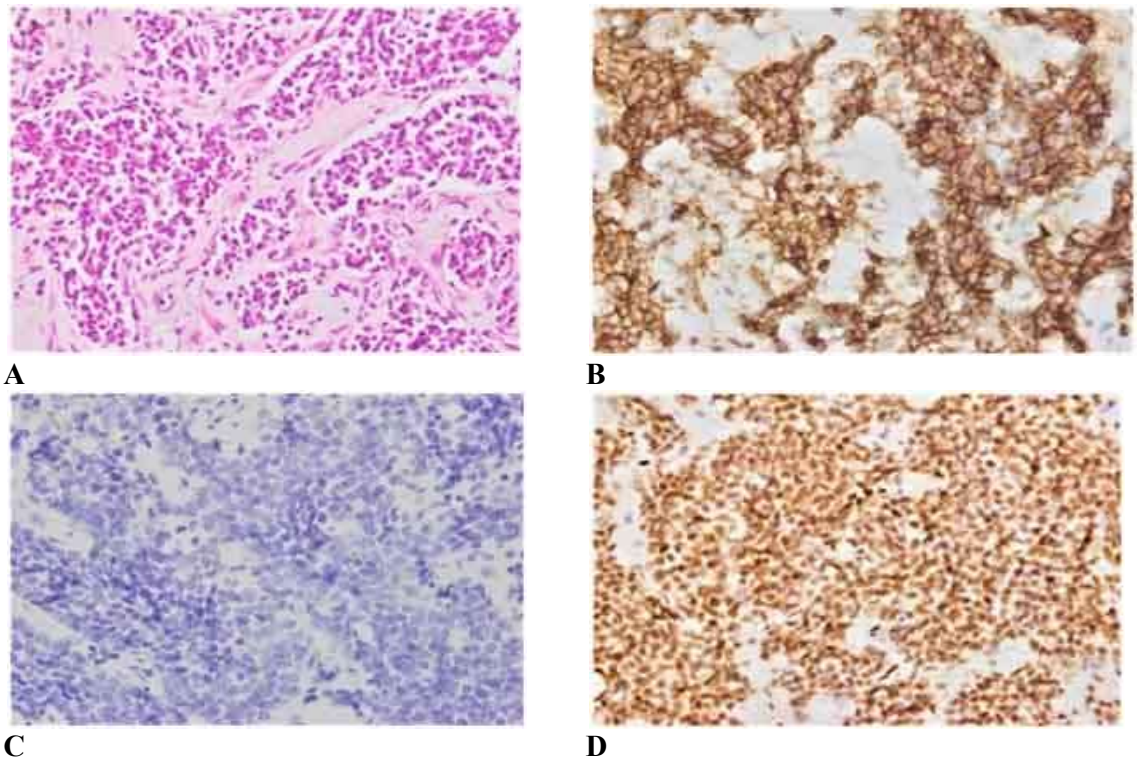

Figure 3. Photomicrograph (400x) of a case of Ewing sarcoma. (A) Hematoxyline and Eosin stain. (B) Diffuse strong membranous immunoreactivity for CD99. (C) Negative reaction for TdT. (D) Diffuse strong nuclear positivity for Cyclin D1

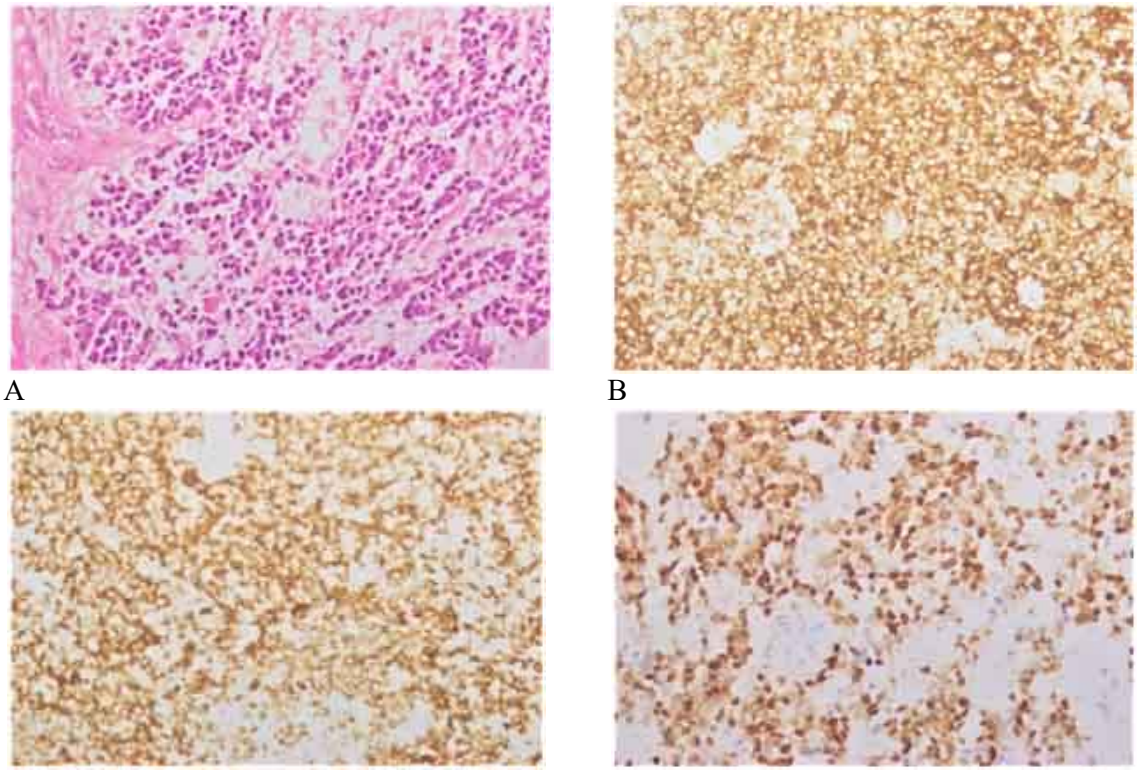

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Figure 4. Photomicrograph (400x) of a case of poorly differentiated neuroblastoma. (A) Hematoxyline and Eosin stain. (B) Positive membranous immunoreactivity for CD56. (C) Strong cytoplasmic reaction for synaptophysin. (D) Diffuse strong nuclear positivity for Cyclin D1.

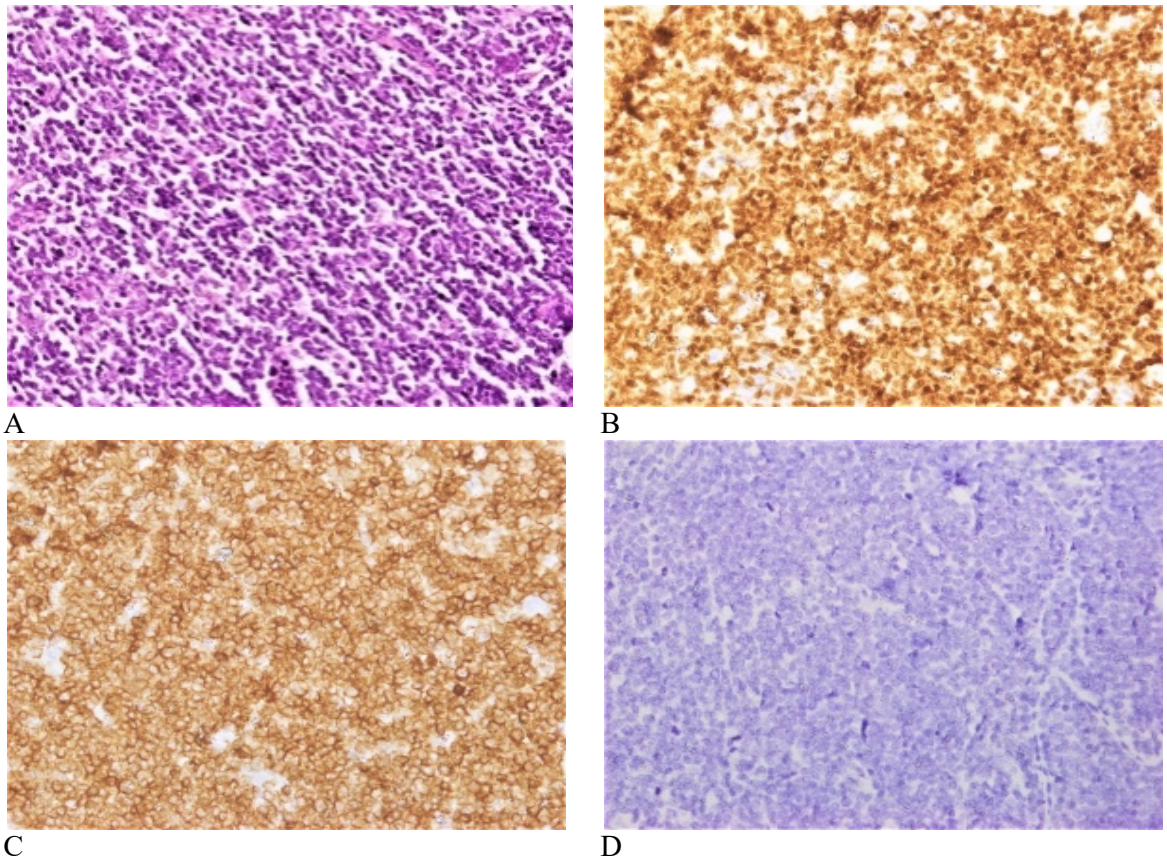

Figure 5. Photomicrograph (400x) of a case of T- Lymphoblastic Lymphoma. (A) Hematoxyline and Eosin stain. (B) Strong nuclear immunoreactivity for TdT. (C) Strong cytoplasmic reaction for CD3. (D) Negative immunoreaction for Cyclin D1.

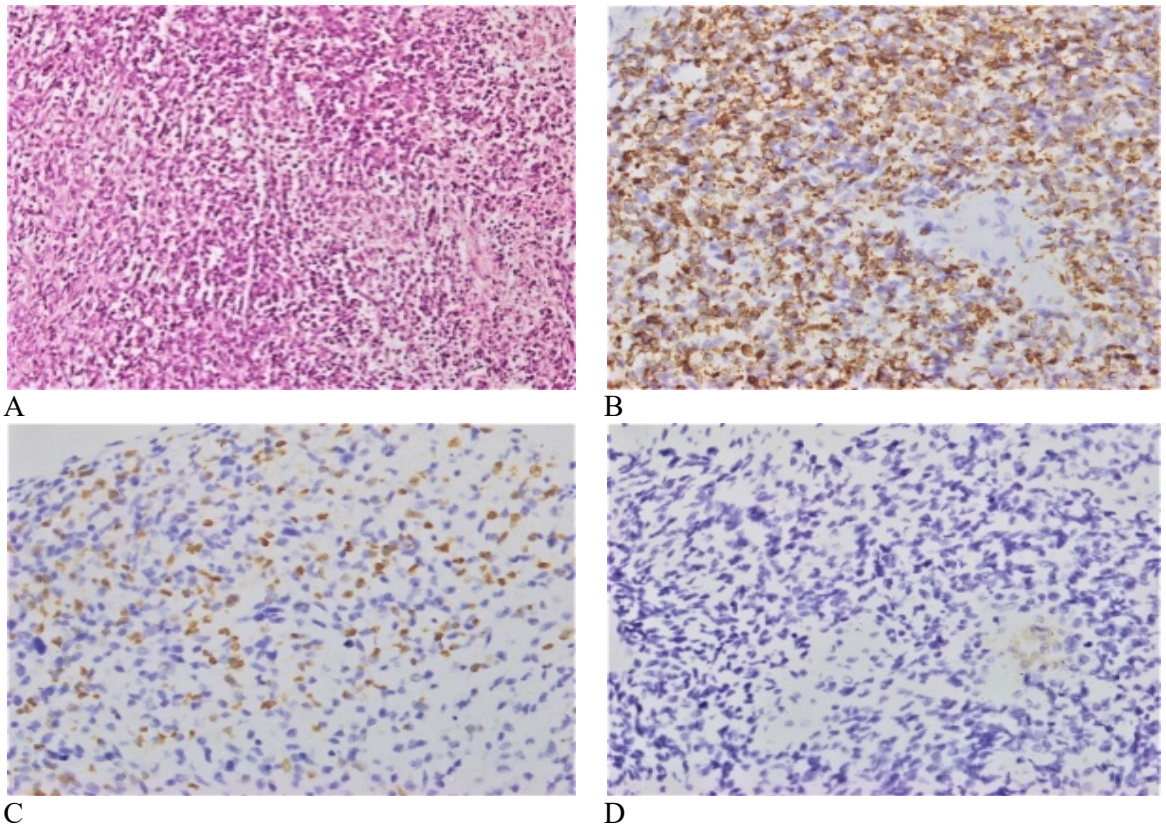

Figure 6. Photomicrograph (400x) of a case of Embryonal Rhabdomyosarcoma. (A) Hematoxyline and Eosin stain. (B) Strong cytoplasmic immunoreactivity for Desmin. (C) Nuclear reaction for Myogenin. (D) Negative immunoreaction for Cyclin D1.

Discussion

In the present study, twenty (31.25%) cases of soft tissue EWS, 15 (23.44%) cases of NB, 14 (21.87%) cases of LBL, 10 (15.62%) cases of RMS, and five (7.81%) cases of WT were selected. All tissue samples were soft tissue in origin and are derived from untreated patients. A total of 20 immunohistochemically confirmed cases of EWS including two metastatic cases were taken for this study. The tumor sites were varied in this study and most tumors (14/20) were derived from surgical biopsies and six cases were from needle core biopsies. The male-to-female ratio was noted as 2:1, with ages ranging from four to 14 years (mean age 10.80 years). EWS is more common in the second decade of life and the second most common bone and soft tissue sarcoma in children.[13]. Histologically tumors are composed of uniform small round blue cells arranged in a vaguely lobular to sheet-like growth pattern with varying degrees of neuroectodermal differentiation. They were strongly and diffusely stained (along the cell membrane of neoplastic cells) with CD99 (Figure 1 (b)). Literature showed that the immunohistochemical profile of EWS includes reactivity for CD99 with a sensitivity ranging from 95-100% and vimentin with a sensitivity ranging from 80-90%.[14,15]

This study showed that Cyclin D1 was over-expressed in all cases of EWS (Fig-3). Seventy percent (14 /20) of cases of

EWS showed diffuse nuclear staining for Cyclin D1, whereas only four (20%) cases were heterogeneously stained and two (10%) cases were focally stained. Fourteen (70%) cases showed strong staining intensity and six (30%) cases were stained with moderate intensity for Cyclin D1. Fifty percent (10/20) of cases showed diffuse staining with strong intensity and 20% (04/20) of cases were diffusely stained with moderate intensity. Out of four (20%) heterogeneously stained cases, two (10%) cases showed strong intensity, whereas the remaining two (10%) cases were moderately stained. Both focally stained cases showed strong intensity for Cyclin D1.

The findings found in some recent studies showed that Cyclin D1 was diffusely expressed (>50% stained neoplastic cells) with strong intensity in the EWS of the pediatric population.[5,11,17] The staining patterns of Cyclin D1 in EWS found in this study were also supported by another recent study showing significant upregulation of Cyclin D1 in EWS as compared to normal tissue by microarray analysis.[18] A recent molecular study explained that EWS–FLI1 interacted with several factors, proteins, and enzymes to direct neoplastic transformation by regulating genes involved in Ewing sarcoma pathogenesis including the cyclin D1 gene *CCND1*.[19]

In this study, neuroblastomas constitute 15 (23.4%) cases, being the second most common tumor noted in the present study. Age at presentation ranged from seven months to six years with a mean age of 2.15 years. The male-to-female ratio was noted as 2:1. Neuroblastoma is the third most common tumor in infancy and childhood is; where 90% of these tumors occur within the first 10 years of life and there is a slight male predominance.[20]

The site of neuroblastoma was varied in the present study where the adrenal gland was the most common site. Based on well-established morphological features and immunohistochemical findings, studied cases of neuroblastoma were classified into three (20.00%) undifferentiated neuroblastoma, eight (53.33%) poorly differentiated neuroblastoma, and two (13.33%) differentiating type neuroblastoma. Two metastatic cases of neuroblastoma were also included in this study. About 60% of neuroblastoma display metastasis at the time of diagnosis; the most common sites were regional lymph nodes, bone, and liver.[21]

The current study showed that Cyclin D1 was overexpressed in all (15) cases of neuroblastomas where 66.67% (10/15) of cases were diffusely stained (>50% stained neoplastic cells) for Cyclin D1, and 93.33% (14/15) of cases showed strong staining intensity for the same marker (Table I). Among the eight (53.33%) cases of poorly differentiated neuroblastoma, five (33.34%) cases were diffusely stained (>50% stained neoplastic cells), two (13.33%) cases were heterogeneously stained (11-50% stained neoplastic cells) and one (6.67%) case was focally stained (1-10% stained neoplastic cells). Out of eight (53.33%) cases of poorly differentiated neuroblastoma, seven (46.67%) cases showed strong (3+) staining intensity (Fig-4), and the remaining one (6.67%) showed moderate (2+) staining intensity. Among three (20.00%) cases of undifferentiated neuroblastoma, two (13.33%) cases were heterogeneously stained with strong intensity; whereas the remaining one (6.67%) was stained in a diffuse pattern with strong intensity. Two (13.33%) cases of differentiating neuroblastoma showed diffuse and strong nuclear reactions for Cyclin D1. Two (13.33%) metastatic neuroblastomas also showed a diffuse nuclear staining pattern for Cyclin D1 with strong intensity.

Some recent studies reported that Cyclin D1 was diffusely overexpressed with

strong intensity in the neuroblastoma of children.[22,23] The cause of Cyclin D1 overexpression was unknown; however, *GATA3* was found to be most consistently correlated with Cyclin D1.[24]
Fourteen immunohistochemically confirmed cases of LBL (TdT positive) were taken for this study. Age at presentation ranged from one to 14 years with a mean age of 8.87 years. The male-to-female ratio is 3.67:1. All the cases of LBL were detected within the lymph node. Eleven (78.57%) cases were T-lymphoblastic lymphoma (T-LBL). Samples were derived from surgical biopsies (100%). The most common NHL among children under 15 years is lymphoblastic lymphoma (LBL); T-cell lymphoblastic lymphomas (T-LBL) are more frequent (85%) than B-cell lymphoblastic lymphoma (B-LBL) (15%).[25]All the cases of LBL irrespective of subtypes were negative for Cyclin D1(Fig-5). Some immunohistochemical studies reported a similar result.[26]

In this study, 10 confirmed cases of rhabdomyosarcoma (RMS) were included of which eight (80%) cases were embryonal rhabdomyosarcoma (ERMS) and two (20%) cases were alveolar rhabdomyosarcoma (ARMS). In the present study, all the cases were positive for desmin, myogenin, and vimentin. In children, embryonal rhabdomyosarcoma is more common (75%) than alveolar rhabdomyosarcoma (16%).[27] The male-to-female ratio was 1.5:1, male children were affected more commonly than females in this study. All cases of RMSs regardless of the subtypes showed negative expression of Cyclin D1 (Fig-6). Some recent studies on malignant SRBCTs obtained similar results.[5,22]

Five cases of Wilms tumor were taken for this study. Patients with WT diagnoses were two females and three males, with ages ranging from two to 10 years (mean age of five years). About 95% of cases of WT occur before 10 years of age.[3] All the cases were derived from surgical biopsies (nephrectomy specimens). All (100%) of the cases showed a triphasic pattern, and diagnoses were confirmed by well-established morphological features without IHC. Most Wilms tumors are readily identified in hematoxylin-eosin sections by distinctive histological features, immunohistochemistry is rarely needed.[28]

Blastemal components of two (40%) cases were focally positive (1-10% stained neoplastic cells) with moderate intensity (2+) for Cyclin D1. In the remaining three (60%) cases, blastemal components were negative for Cyclin D1. Epithelial components of all cases showed diffuse expression with strong intensity for Cyclin D1.

A few recent studies showed a blastemal component in 40% of cases (6 out of 15) of Wilms tumor focally (1-10% stained neoplastic cells) stained for Cyclin D1, whereas 60% of cases (9 out of 15) showed negative reaction for the same antibody.[5,12]

Conclusion

The present study suggests that cyclin D1 can be exploitable as a diagnostic adjunct to conventional markers in confirming the diagnosis of Ewing sarcoma or Neuroblastoma. Its use in routine practice also might be helpful for those cases of SRBCTs with undifferentiated morphology that is difficult to diagnose following the application of the conventional markers. It is important to differentiate the specific lineage of a neoplasm exhibiting small round cell morphology for better guidance of therapeutic decisions and to predict the prognostic outcome.

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