ORIGINAL ARTICLE

Histopathological Spectrum and Immunohistochemistry Expression of Pediatric Solid Malignant Tumors: An Observational Study

Bhumika Patel, Jagadale Kunda, Reena Bharadwaj

Abstract

Introduction: The major leading cause of disease-related mortality in children is childhood cancer. It can be difficult to make an accurate diagnosis using only histopathology due to overlapping features and similar histomorphological appearances in various cancers. Immunohistochemistry (IHC) markers can help distinguish tumors that show similar histomorphological features. **Aims & Objectives:** This study was undertaken to evaluate the histopathological spectrum of pediatric solid malignant neoplasms with IHC and to assess the role of IHC in pediatric solid malignant tumors. **Study Design:** An observational study of resected malignant solid tumors in the pediatric population including Gross and Histopathological examination was conducted. **Materials and methods**: 55 samples were collected for five years. All samples were processed for routine histopathology and IHC was done on the majority of these samples. **Results:** The top five categories of tumors were retinoblastomas, lymphomas, neuroblastomas, bone and soft tissue tumors, and renal and reproductive system tumors. The mean age is 6.6 years and most commonly presented in 0-5 years of age constituting 30 cases. 35 cases seen were in male patients, and 20 in female patients. IHC was performed on 44 cases for confirmation of diagnosis. For the rest of the 11 cases, the histopathological study is the gold standard for diagnosis. **Conclusion:** An integrated approach including Histomorphological features is required for provisional diagnosis and IHC is necessary for final diagnosis and further characterization.

Key Words

Pediatric, Solid Tumours, Malignancy, Immunohistochemistry

Introduction

30% of all pediatric cancers are solid tumors. The most common of these are likely to be brain tumors, neuroblastoma, and rhabdomyosarcoma[1]. With the increasing incidence of childhood cancers worldwide, cancer represents the leading cause of disease-related mortality in children[2]. Studies suggest that 8–10% of all cancer cases in children are thought to be predisposed due to genetic factors[3]. Histopathological analysis offers invaluable information about the characteristics of the tumorin pediatric patients[4].

However, it can be difficult to make an accurate diagnosis using only conventional histopathology because different types of pediatric solid malignancies have

Department of Pathology, Bharati Vidyapeeth, Deemed to be University Medical College, Pune, India

Correspondence to: Dr. Bhumika Patel, B/801,Seventh Bliss,Nr Vishnudhara Gaedens, Gota- Jagatpur Road, Gota, Ahmedabad, 382481, Gujrat

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overlapping features and differing histological appearances[5]. Immunohistochemistry (IHC) is a powerful adjunctive tool in the diagnostic assessment of pediatric solid malignant neoplasms. By using particular antibodies, IHC finds and examines the expression of different proteins or antigens within tumor tissues. IHC can help to distinguish between tumors with similar histology, identify particular tumor subtypes, and determine prognosis[6].

Despite its widespread use, there is a paucity of comprehensive studies exploring the histopathological spectrum of pediatric solid malignant neoplasms using IHC techniques.

The current study focuses on finding the spectrum and importance of in all biopsies and resected specimens in pediatric solid malignant tumors. This aids in faster tumor diagnosis, classification, and staging for appropriate therapy selection and maximizes cure rates to improve patient outcomes.

This study aims to evaluate the histopathological spectrum of pediatric solid malignant neoplasms with IHC. The primary objective of this study is to evaluate the histopathological spectrum of solid malignant neoplasms in the pediatric age group. The secondary objective is to determine the role of IHC in the same group of tumors.

Materials and Methods

Sample Collection:

A total of 55 resected specimens and biopsies of solid malignant tumors in the pediatric age group were studied within 5 years in the pathology department of the institute under the current study. All histopathologically benign tumors in the pediatric age group and inadequate samples were excluded from this study. This sample size was calculated based on samples received in one year and samples collected over five years in our institute. All specimens were collected in 10% formalin. For retrospective cases, relevant paraffin-embedded tissue blocks were retrieved from the departmental archives, and details of the cases were noted from test request forms.

Fresh slides were prepared from the collected specimens and stained for examination. Both the gross specimens of prospective cases and the preserved retrospective cases were examined to assess their gross details.

Histopathological examination was performed on all specimens, following the CAP protocols, to evaluate the relevant prognostic parameters. The assessment included the examination of cellular morphology, tumor differentiation, and architectural patterns.

To investigate further, paraffin tissue sections were prepared on pre-coated slides specifically for immunohistochemistry (IHC). The IHC staining was carried out using standard protocols, which involved the application of specific antibodies to detect and analyze the expression of proteins or antigens within the tumor tissues.

The collected data was coded and entered into a Microsoft Excel sheet. The statistical analysis was performed using SPSS (Statistical Package for Social Sciences) version 26.0 software. This study was approved by the ethical committee under reference number BVDUMC/IEC/114 on August 20, 2022.

Results

The study encompassed 55 pediatric solid malignant neoplasm patients, reflecting a population primarily composed of young children (age 17 years). Additionally, the median age of 48 months suggests that many of the cases involved children around the ages of 3 to 4 years. The minimum age within the sample was 3 months, while the maximum age was 17 years.

Table 1 shows the age-wise distribution of pediatric solid malignant tumors, which shows the maximum number of tumors found between 0 and 5 years of age.

In terms of gender distribution, there were 35 male patients and 20 female patients, demonstrating a higher prevalence of males within the study population.

Table 1: Age Group-wise Distribution of Solid Malignant neoplasms

Age (years)	Number of cases
0 to 5	30
6 to 10	8
11 to 15	13
16-17	4

In the distribution of solid malignant neoplasms in pediatric age groups, for female patients, the mean age was 69.78 ± 62.59 months. In comparison, male patients had a mean age of approximately 73.23 ± 65.16 months.

WHO Pediatric Solid Tumor Classification

The results in **Table 2** showcase the distribution of various pediatric solid malignant neoplasms based on diagnostic groups and histologic types, classified according

Table 2: Classification of Pediatric Solid Tumors According to WHO in the Study Population

Diagnostic group	Histologic type	No of cases	Percentage (%)	Total
Lymphoma	T cell lymphoblastic lymphoma	5	9.09	6
	ALK-positive anaplastic large-cell lymphoma	1	1.82	
Bone and Soft tissue tumor	Ewing's sarcoma	6	10.91	15
	Rhabdomyosarcoma	2	3.64	
	Synovial sarcoma	1	1.82	
	Osteosarcoma	6	10.91	
Eye tumor	Retinoblastoma	10	18.18	10
Renal and reproductive system	Wilms tumor	4	7.27	10
	Immature teratoma	5	9.09	
	Juvenile granulosa cell tumor	1	1.82	
Digestive system	Hepatoblastoma	3	5.45	4
	Appendiceal neuroendocrine tumor	1	1.82	
Head and neck tumors	Undeferential Nasopharyngeal carcinoma	1	1.82	1
CNS tumors	Astrocytoma	2	3.64	3
	Medulloblastoma	1	1.82	
Neuroblastoma		6	10.91	6

to the World Health Organization (WHO) standards. In the lymphoma group, T-cell lymphoblastic lymphoma is the most prevalent type, with 5 (9.09%) cases, while ALK-positive anaplastic large cell lymphoma has 1 (1.82%) case. The bone and soft tissue tumor group is dominated by Ewing's sarcoma and osteosarcoma, each with 6 (10.91%) cases, indicating a higher prevalence of these types, while rhabdomyosarcoma and synovial sarcoma had 2 (3.64%) and 1 (1.82%) case(s), respectively. The eye tumor group consists solely of retinoblastoma, accounting for all 10 (18.18%) cases. In the renal and reproductive system group, Wilms tumor and immature teratoma are the most common types, with 4 (7.27%) and 5 (9.09%) cases, respectively, while juvenile granulosa cell tumor is less common, with only 1 (1.82%) case. The digestive system group predominantly features hepatoblastoma, with 3 (5.45%) out of 4 cases, while appendiceal neuroendocrine tumor is less frequent, with just 1 (1.82%) case. The head and neck tumor group contains only 1 case of undifferentiated nasopharyngeal carcinoma, suggesting a lower prevalence of this type. Within the CNS tumors group, astrocytoma and medulloblastoma are represented by 2 (3.64%) and 1 (1.82%) case(s), respectively, indicating these types are not as common in this sample. Lastly, the neuroblastoma group has a total of 6 (10.91%) cases, suggesting it is an important condition to consider in pediatric solid malignant neoplasms.

Immunohistochemistry Findings

Among the 55 pediatric patients examined with solid malignant neoplasms, the following revelations were noted:

Five cases of T cell lymphoblastic lymphoma show TdT positivity, which shows lymphoblastic lymphoma, and CD3 and CD5 positivity, which show the tumor's T cell origin. One case of ALK-positive ALCL shows strong positivity for ALK.

The IHC findings included six cases (10.91%) of neuroblastoma showing synaptophysin, chromogranin, and CD56 positivity. Also, the 4 cases (7.27%) of Wilm's tumor show WT1, and the 3 cases (5.45%) of hepatoblastoma show Hepar1 positivity. In the 2 (3.64%) astrocytoma cases, IHC revealed the absence of ATRX and the presence of mutated IDH, identifying a specific molecular subtype (IDH mutant) of astrocytoma that might

not have been distinguishable by histopathology alone. One case (1.82%) of juvenile granulosa cell tumor (JGCT) shows inhibin and vimentin, six cases (10.91%) of osteosarcoma show SATB2, and five cases (9.09%) of immature teratoma show SALL4 positivity.

One (1.82%) case was diagnosed as an appendicular neoplasm on histopathology. However, after chromogranin and synaptophysin positivity, it was confirmed as an appendiceal neuroendocrine tumor. For one (1.82%) case, the differential diagnosis was given as rhabdomyosarcoma or synovial sarcoma. It showed negativity for Desmin and MyoD1 and positivity for TLE1. So, the final diagnosis was given as synovial sarcoma. Two (3.64%) cases were diagnosed as rhabdomyosarcoma. **Figure 1** shows their histopathological and immunohistochemistry findings.

For another (1.82%) case, the differential diagnosis was given as nasopharyngeal carcinoma, or NHL. It showed positivity for p63 and CK but negativity for LCA and ALK. Therefore, the final diagnosis was given as nasopharyngeal carcinoma.

Six cases (10.91%) were diagnosed as poorly differentiated malignancies on histopathology. All these cases were positive for CD99 and NKX2.2 **(Figure 2)**. Other markers such as SMA, synaptophysin, LCA, and Desmin were negative for all cases. Therefore, the final diagnosis for all six cases was given as Ewing's sarcoma.

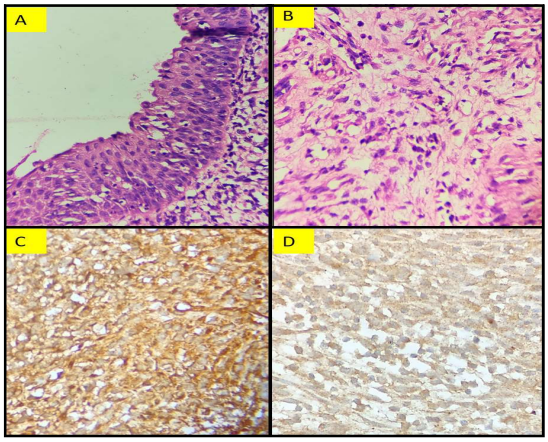

Fig 1: (A) Low power of H&E stain in rhabdomyosarcoma shows the cambium layer; (B) High power of H&E stain shows many cells are maturing into spindle-shaped cells with oval nuclei and ample eosinophilic cytoplasm. (C) Immunohistochemistry of MyoD1 shows strong positivity. (D) Immunohistochemistry of Desmin shows deemed positivity.

Cases Where Immunohistochemistry Was Not Necessary.

In the context of the 55 pediatric patients with solid malignant neoplasms, there were instances where IHC was deemed unnecessary due to the robustness of the histopathological diagnosis alone. Specifically, 10 cases (18.18%) of retinoblastoma and 1 case (1.82%) of medulloblastoma did not require IHC for confirmation.

Out of the total cases examined, 44 cases (80%) benefited from the use of IHC, while in 11 cases (20%), IHC was deemed unnecessary for the diagnosis or characterization of the condition under study. These findings summarize the necessity of immunohistochemistry (IHC) among the study population.

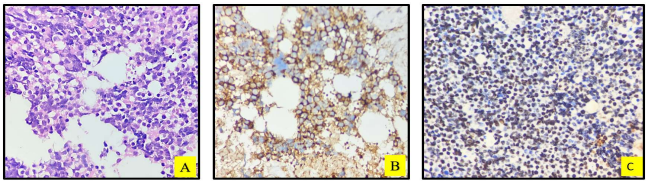

Figure 2: (A) The High Power of Ewing's Sarcoma Shows Small Round Blue Cells with a high N:C Ratio, Atypia, and Mitotic Figures. (B) Immunohistochemistry Shows CD99 positivity;)C) Immunohistochemistry Shows NKX2.2 positivity.

Table 3: Spectrum of Pediatric Solid Tumors in Similar Studies.

Histologic type	Banerjee et al[10]	Current study
Lymphoma	25.92%	10.9%
Bone and soft tissue tumor	24.82%	27.2%
Renal and gonadal tumors	12.2%	18.1%
Brain tumors	15.32%	0.54%
Retinoblastoma	8.7%	18.1%
Neuroblastoma	4.5%	10.9%
Others	8.5%	14.26%

Discussion

Various studies studying pediatric solid tumors were compared to our current study. The majority of the cases in the current study are in the age group of 0–5 years. These findings are consistent with Dewani *et al*[7], while they differ from the findings of Jain *et al*.[8]. In this study, tumors are more common in males, which is similar to the findings reported by Miller *et al.*[9].

Table 4: Comparing the Immunohistochemistry of Our Study with a Similar Study

Tumor	Similar study	Findings in the respective study	Findings in our study
T cell lymphoblastic lymphoma	Cortelazzo et al.[11]	TdT in all cases CD3 and CD5 are positive variables.	TdT, CD3 and CD5 positive in all cases
ALK-positive anaplastic large-cell lymphoma	Kaseb et al. [12]	ALK and CD30 positive in all cases	ALK and CD30 were positive in all cases
Ewing's sarcoma	Hung et al.[13]	NKX2.2 positivity in 92% of cases	NKX2.2 and CD99 positivity in all cases
	Chinchilla-Tábora et al. [14]	CD99 positivity in 100% cases	
Rhabdomyosarcoma	Tuohy et al.[15]	MyoD1 is positive in all cases. Desmin is positive in 81.25% of cases.	MyoD1 and Desmin were positive in all cases
Synovial sarcoma	El Beaino et al.[16]	TLE1 shows positivity in all cases.	TLE1 shows positivity in all cases
Osteosarcoma	Hornick et al.[17]	SATB2 positive in all cases	SATB2 positive in all cases
Wilms tumor	Goyal S. et al.[18]	WT1 positivity in all cases	WT1 positivity in all cases
Immature teratoma	Mei et al.[19]	SALL4 positivity in 78% of cases	SALL4 positivity in all cases
Juvenile granulosa cell tumor	Nofech-Mozes et al.[20]	Inhibin positivity in all cases	Inhibin positivity in all cases
Hepatoblastoma	No study done for hepatoblastoma. Takahashi et al.[21] study shows positivity for Hep Par 1 in all hepatocellular carcinoma		
Appendiceal neuroendocrine tumor	Abreu et al.[22]	Synaptophysin and chromogranin positive in all cases	Synaptophysin and chromogranin positive in the only case
Undeferential Nasopharyngeal carcinoma	Mremi et al.[23]	Positive for CK and negative for CD45 (LCA) in all cases	Positive for p63 and CK Negative for LCA and ALK
Astrocytoma	Camelo-Piragua et al. [24]	IDH mutant positive for all cases	IDH mutant positive and ATRX negative for all cases
Neuroblastoma	Brook et al.[25]	Synaptophysin, chromogranin, and CD56 were positive in all cases	Synaptophysin, chromogranin, and CD56 were positive in all cases

The most common histologic type of tumor is bone and soft tissue tumors, followed by retinoblastoma. While other studies predominantly showed lymphomas[10], as shown in **Table 3.**

Table 4 shows a comparison of the IHC findings of our study to those of various similar studies.[11-25]

Pediatric solid malignant tumors differ from adult solid malignant tumors in terms of their histology, clinical presentation, and prognosis. Therefore, it is crucial to obtain an accurate diagnosis as soon as possible to improve the chances of a child's survival. When diagnosing these tumors with a similar histological morphology, immunohistochemistry is crucial. This study gave information about specific IHC markers for their respective tumors.

IHC was crucial not only for narrowing down the differential diagnoses suggested by histopathology but also for confirming the final diagnoses in several cases. This comprehensive approach ensures precise identification of the tumor types, which is vital for

determining the appropriate therapeutic strategies and improving prognostic outcomes in pediatric oncology.

Conclusion

In the context of the 55 pediatric patients with solid malignant neoplasms examined in this study, the use of immunohistochemistry (IHC) proved to be indispensable for achieving accurate diagnoses. Initially, differential diagnoses were suggested based on histopathological examination alone, which involves the microscopic evaluation of stained tissue sections to identify the cellular characteristics of the tumors. However, due to the overlapping histological features among various types of neoplasms, IHC was essential to definitively identify and confirm the specific tumor types. The current study shows that IHC is helpful in cases where differentials were given on a histopathologic study and diagnoses were confirmed on IHC. An integrated approach including histomorphological features and IHC is required for a final diagnosis and further characterization. It is extremely important to further classify tumors biologically, using cytogenetics or molecular studies to help improve patient outcomes.

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References

1. Kline NE, Sevier N. Solid tumors in children. Journal of Pediatric Nursing 2003; *18*(2), 96–102.
2. Zahnreich S, Schmidberger H. Childhood Cancer: Occurrence, Treatment and Risk of Second Primary Malignancies. Cancers *2021; 13*(11): 2607.
3. Gröbner SN, Worst BC, Weischenfeldt J, Buchhalter I, Kleinheinz K, Rudneva VA,et al. The landscape of genomic alterations across childhood cancers. Nature *2018; 555*(7696): 321–27.
4. Ahmed A A, Abedalthagafi M. Cancer diagnostics: The journey from histomorphology to molecular profiling. Oncotarget 2016; *7*(36): 58696–708.
5. Ferreira-Facio CS, Milito C, Botafogo V, Fontana M, Thiago LS, Oliveira E, et al. Contribution of multiparameter flow cytometry immunophenotyping to the diagnostic screening and classification of pediatric cancer. PloS one 2013; *8*(3), e55534.
6. Hawes D, Shi SR, Dabbs DJ, Taylor CR, Cote RJ. Immunohistochemistry. Modern Surgical Pathology 2009; 48–70.
7. Dewani G., Tandon P L, Ghooi A M, Jain PK. Malignant tumours of infancy and childhood. Indian J Surg *1972;34:*460-8.
8. Jain KK, Mathur GP, Srivastava J R. Malignancy in childhood: A clinico-pathological study of 45 cases. Indian journal of pediatrics *1975; 42*(326): 61–66.
9. Miller RW, Young J L, Novakovic B. Childhood cancer. Cancer 1995; *75*(1 Suppl): 395–405.
10. Banerjee CK, Walia BN, Pathak IC. Pattern of neoplasms in children. Indian Journal of Pediatrics *1986; 53*(1), 93–97.
11. Cortelazzo S, Ferreri A, Hoelzer D, Ponzoni M. Lymphoblastic lymphoma. Crit Rev Oncol Hematol. 2017 ;113:304-17.
12. Kaseb H, Mukkamalla SKR, Rajasurya V. Anaplastic Large Cell Lymphoma. [Updated 2023 Jul 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing;2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537150/
13. Hung YP, Fletcher CD, Hornick JL. Evaluation of NKX2-2 expression in round cell sarcomas and other tumors with EWSR1 rearrangement: imperfect specificity for Ewing sarcoma. Mod Pathol 2016;29:370–80.
14. Chinchilla-TáboraLM, Ortiz Rodríguez-Parets J, González Morais I, Sayagués JM, Ludeña de la Cruz MD. Immunohistochemical Analysis of CD99 and PAX8 in a Series of 15 Molecularly Confirmed Cases of Ewing Sarcoma. Sarcoma 2020 ; 2020(1):7192347
15. Tuohy JL, Byer BJ, Royer S, Keller C, Nagai-Singer MA, Regan DP, et al. Evaluation of Myogenin and MyoD1 as Immunohistochemical Markers of Canine Rhabdomyosarcoma. Vet Patho 2021 ;58(3):516-26.
16. El BeainoM, Jupiter DC, Assi T, Rassy E, Lazar AJ, Araujo DM, Lin PP. Diagnostic value of TLE1 in synovial sarcoma: a systematic review and meta-analysis. Sarcoma 2020 29;2020.
17. Hornick JL. Novel uses of immunohistochemistry in the diagnosis and classification of soft tissue tumors. Modern Pathology. 2014 ;27:S47-63.
18. Goyal S, Mishra K, Sarkar U, Sharma S, Kumari A. Diagnostic utility of Wilms' tumour-1 protein (WT-1) immunostaining in paediatric renal tumours. Indian J Med Res 2016 ;143(Supplement):S59-S67.
19. Mei K, Liu A, Allan RW, Wang P, Lane Z, Abel TW, et al. Diagnostic utility of SALL4 in primary germ cell tumors of the central nervous system: a study of 77 cases. Modern pathology 2009 ;22(12):1628-36.
20. Nofech-Mozes S, Ismiil N, Dubé V, Saad RS, Khalifa MA, Moshkin O, Ghorab Z. Immunohistochemical characterization of primary and recurrent adult granulosa cell tumors. International journal of gynecological pathology. 2012 ;31(1):80-90.
21. Takahashi Y, Dungubat E, Kusano H, Ganbat D, Tomita Y, Odgerel S, Fukusato T. Application of immunohistochemistry in the pathological diagnosis of liver tumors. International journal of molecular sciences. 2021;22(11):5780.
22. Abreu RP. Appendiceal neuroendocrine tumors: approach and treatment. Journal of Coloproctology (Rio de Janeiro). 2018 ;38(4):337-42.
23. Mremi A, Yahaya JJ, Abraham ZS, Mwakigonja AR. The role of a minimum immunohistochemical antibody panel in confirming undifferentiated nasopharyngeal carcinoma: a cross-sectional study at the Muhimbili National Hospital, Dar-es-Salaam, Tanzania. Nigerian Medical Journal 2019 ;60(6):279-84.
24. Camelo-PiraguaS, Jansen M, Ganguly A, Kim JC, Louis DN, NuttCL. Mutant IDH1-specific immunohistochemistry distinguishes diffuse astrocytoma from astrocytosis. Acta Neuropathol 2010;119(4):509-11.
25. Brook FB, Raafat F, Eldeeb BB, Mann JR. Histologic and immunohistochemical investigation of neuroblastomas and correlation with prognosis. Hum Pathol 1988;19(8):879-88.