https://doi.org/10.29309/TPMJ/2026.33.05.10240 www.theprofesional.com

ORIGINAL ARTICLE

Molecular subtyping of medulloblastoma on the basis of immunohistochemistry at Chughtai Institute of Pathology, a single-center experience.

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ABSTRACT… Objective: To determine the frequency of medulloblastoma subtypes using IHC markers (β-catenin, YAP1, TP53). **Study Design:** Cross-sectional study. **Setting:** The Chughtai Institute of Pathology. **Period:** June 2024 to June 2025. **Methods:** Twenty-one new diagnoses of medulloblastoma were included and H&E staining for histological variants was processed. Then immune staining using β-catenin, YAP1, and TP53 was performed. The staining results were used to classify medulloblastoma into its subtypes. Chi-square testing and descriptive statistics were used to conduct the statistical analysis. **Results:** Classic variant was found to be the most common histological type of medulloblastoma. The most frequently detected subtype of medulloblastoma was the Group 3/4 (76.2%), followed by the SHH subtype (23.8%). No WNT subtype was detected. The identification of WNT tumors by nuclear β-catenin negativity and the identification of desmoplastic/nodular histology by the expression of YAP1 were significantly associated (p=0.021). **Conclusion:** IHC-based molecular subtyping is a cost-effective and reliable method for classifying medulloblastomas in resource limited situations. This can help to individualize therapies for medulloblastomas patients.

Key words: Beta Catenin, Immunohistochemistry, Medulloblastoma, Molecular Subtyping, Neoplasms, Sonic Hedgehog Signaling Pathway, Wnt Signaling Pathway.

Article Citation: Rana HR, Rathore Z, Jahangir F, Afzal S, Zaman S, Chughtai AS. Molecular subtyping of medulloblastoma on the basis of immunohistochemistry at Chughtai Institute of Pathology, a single-center experience. Professional Med J 2026; 33(05):869-874. https://doi.org/10.29309/TPMJ/2026.33.05.10240

INTRODUCTION

Medulloblastoma is the most commonly diagnosed malignant brain tumor among children, comprising almost one-fifth of all pediatric central nervous system tumors. It primarily occurs in children, but adult cases, though less common, account for approximately 36% of all medulloblastoma cases, presenting unique diagnostic challenges.[1] The development of new molecular pathology techniques over the last several years has demonstrated that medulloblastomas comprise various genetically distinct groups rather than having a single origin; therefore, based on this knowledge, current molecular categorization for medulloblastoma uses four primary groups (subtypes) including: WNT, SHH, group 3, and group four.[2,3]

Each of these subtypes is characterized by unique genetic changes as well as differences in pathology, geographical location, and clinical outcomes[4,5] Group WNT tumors are the rarest subtype but represent the best prognosis for survival. They typically contain expression of nuclear β-catenin and show loss of chromosome 6, detectable with immunohistochemistry[6] Treatment-related morbidity for patients with WNT medulloblastoma is lower since that population achieves long-term survival rates with less aggressive therapies compared to other major subtypes[7] The SHH subtype has aberrant activation of the Sonic Hedgehog pathway due to mutations in genes such as PTCH1, SUFU, and GLI.[1,8]

This group represents many different clinical features and outcomes based on the age of the patient; infants experience different clinical course outcomes than adults. The sequencing of the methylatome on SHH tumors provided further complexity and information on SHH[9] The majority of non-WNT/non-SHH medulloblastomas are found in groups 3 and 4, with group 3 being associated with the worst prognosis. In group 3, MYC amplification and large cell/anaplastic histology are common[10,11] Although group 4 is more prevalent than group 3, its molecular background remains poorly understood. Group 4 tumors generally show whole chromosome changes and have mutations in chromatin-modifying genes; therefore, methylation profiling can further stratify prognosis.[12,13] The intertumoral heterogeneity of the two subgroups shows that precise molecular classification is

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Article received on:
10/11/2025
Date of revision:
17/01/2026
Accepted for publication:
17/01/2026

necessary for stratifying risk and deciding upon the most effective treatment.[14-15]

The finding of molecular subgroups has reshaped the clinical management of medulloblastoma. Subgrouping has become a vital prognostic tool for developing individualized therapy, as it allows for personalized treatment through risk-adapted chemotherapy and radiotherapy plans[16-17] Although there are challenges related to limited resource environments, molecular subgrouping based on immunohistochemistry provides an inexpensive and readily available way to classify these tumors and generate prognosis comparable to more sophisticated genomic tests[18,19]

In light of this evidence, the primary goal of the present study was to investigate the use of immunohistochemistry (β-catenin, synaptophysin, p53, and YAP-1) and Special stains (Reticulin) in determining the subtypes of medulloblastoma at the Chughtai Institute of Pathology. This study will present data that describes how frequently each of the outlined subtypes is found in our local population. These data will aid in generating rational methods for risk stratification, individualized treatment strategies, and future aims of developing precise therapy for patients in our region.

METHODS

Our cross-sectional prospective study of the pathological features of newly diagnosed medulloblastomas was conducted at the Department of Histopathology, Chughtai Institute of Pathology in Lahore, Pakistan, from June 2024 to June 2025. Ethical approval to conduct this study was obtained from the Institute's Institutional Review Board (IRB letter number CIP/IRB/1356 and Dated:12-12-2025). All research procedures were carried out in accordance with the Declaration of Helsinki. A total of 21 patients with newly diagnosed medulloblastoma were enrolled, including both pediatric (n=16) and adult cases (n=5), with ages ranging from 2 to 37 years. The patients with newly diagnosed medulloblastoma were diagnosed by a histopathologist through examining their biopsy or resection specimens. Any tumour that had insufficient tissue for performing immunohistochemistry, poorly fixed tissue specimens, or tumours later diagnosed as high-grade gliomas were excluded from the study to ensure accurate determination of each patient's molecular sub-group. Special staining using Reticulin was performed on formalin-fixed, paraffin-embedded tumor sections using the standard Gordon and Sweet method to highlight the reticulin fibers, complementing immunohistochemical analysis for molecular subtyping of medulloblastoma. An accurate representation of all tumours suitable for immunohistochemical analysis was accomplished through this strict selection process.

The tissue specimens of the medulloblastoma tumors were acquired through surgical resection or biopsy. Fixation of all specimens in 10% neutral buffered formalin was immediate and lasting 24–48 hours to maintain tissue architecture and antigenicity of each specimen. Routine processing of each specimen after fixation to produce a paraffin wax block, using paraffin embedded sections cut to 4 µm thickness made it possible to perform a haemotoxylin and eosin (H&E) stain for confirmation of the pathological diagnosis of medulloblastomas, and using the WHO classification guidelines established by Louis et al, 2021, for classifying the different tumour variants. For performing immunohistochemical (IHC) staining, in addition to the 4 µm thick H&E stained sections, 4 µm thick slides of the paraffin wax embedded tissue sections where prepared on poly-L-lysine coated glass slides. Paraffin wax embedded tissue sections were deparaffinized in xylene and rehydrated through a graded ethanol series and heat induced epitope retrieval was performed using citrate buffer (pH 6.0) in a microwave oven for 15 minutes. Blocking of endogenous peroxidase activity occurred using a 3% hydrogen peroxide solution for 10 minutes at room temperature.

According to the manufacturer's recommendations, (for β-catenin, YAP1, Synaptophysin, and TP53) primary antibodies were diluted and then applied with a 1-hour incubation period at room temperature. After primary antibody incubation, a polymer-based detection system (secondary) was used, and the antigen-antibody complex was visualized by use of 3,3′-diaminobenzidine (DAB). The slides were counterstained with hematoxylin, dehydrated, cleared, and mounted using coverslips. Evaluation of β-catenin expression for nuclear staining defined WNT subgroup medulloblastomas, while YAP1 expression defined SHH subgroup medulloblastomas, and TP53 provided further stratification of SHH subgroup of tumours where the patient has been identified at high risk. Scoring of staining intensity and the percentage of positive tumour cells from the IHC analysis were performed by two independent pathologists who evaluated all slides. If there was any discordance in interpretation, the two pathologists re-evaluated the case together until they reached a consensus. Sub-classification of medulloblastomas into the four subtypes WNT, SHH, Group 3 and Group 4 were performed based on the IHC algorithms that we established, and these sub-types were found to be very reliable proxies for their genetic classification.

This collected demographic and clinical information for each patient including age, sex and the type of tumour

from medical records. Quantitative variables were summarized as mean and standard deviation (SD); Categorical variables (e.g. sex) were summarized as frequency and percentage. Chi-square tests were used to assess whether there was an association between IHC markers and histological subtypes of medulloblastoma. A p-value of 0.05 was determined to be statistically significant and all statistical analyses performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 21 patients with newly diagnosed medulloblastoma were included in the study. The majority (16, 76.2%) were pediatric cases aged 2-17 years, while 5 patients (23.8%) were adults aged 18–37 years, highlighting the occurrence of medulloblastoma beyond childhood. Of the total cohort, 13 (61.9%) were males and 8 (38.1%) were females, yielding a male-to-female ratio of 1.6:1. The mean age of the cohort was 9.2 ± 4.7 years, with an age range of 2 to 37 years.

TABLE-I

Demographic and clinical characteristics of study participants.

Variable	n (%) or Mean ± SD
Total patients	21
Age (years)	9.2 ± 4.7 (range 2–37)
Pediatric patients (2-17 years)	16 (76.2%)
Adult patients (18-37 years)	5 (23.8%)
Gender	Male: 13 (61.9%) Female: 8 (38.1%)

Histological Variants

Histopathological examination revealed that the classic variant was the most common subtype, observed in 16 cases (76.2%), while the desmoplastic/nodular variant accounted for 5 cases (23.8%).

TABLE-II

Histological variants and molecular subtypes of medulloblastoma

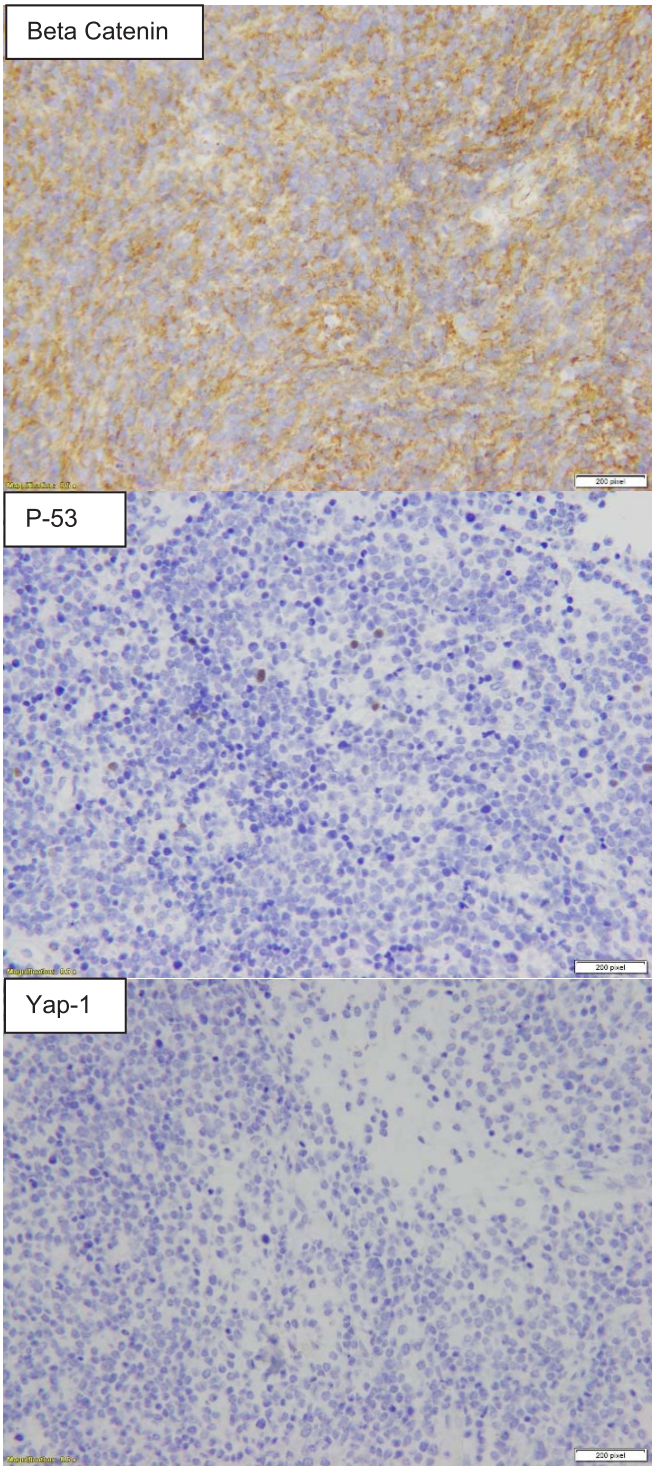
Histological Variant	Number of Cases (%)	Molecular Subtype	Number of Cases (%)
Classic	16 (76.2%)	SHH	5 (23.8%)
Desmoplastic/ Nodular	5 (23.8%)	Group 3/4	16 (76.2%)
–	–	WNT	_

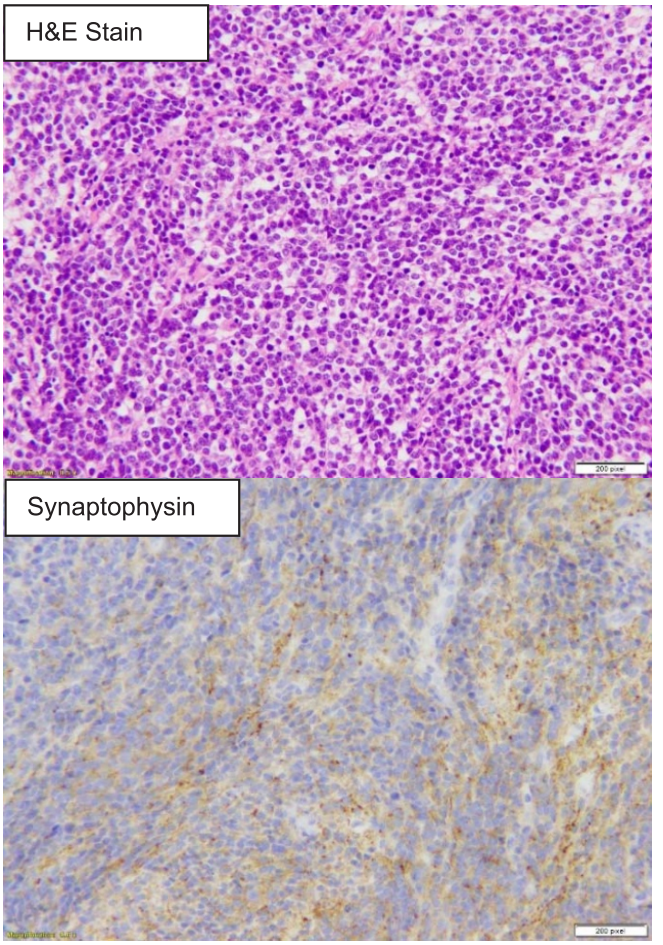
Molecular Subtyping Based on Immunohistochemistry

Immunohistochemical analysis allowed classification of medulloblastomas into the four main molecular subgroups (Figure-1).

FIGURE-1

Immunohistochemistry showed βcatenin negative, synaptophysin positive, p53 wild-type staining pattern, and YAP-1 negative, supporting the diagnosis of a non-WNT, non-SHH medulloblastoma."





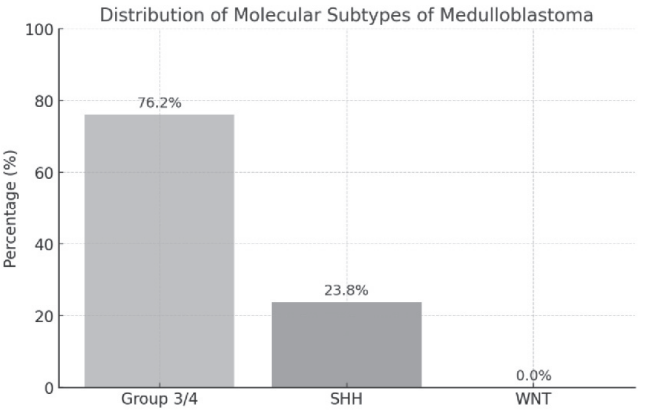
The most frequently detected subtype of medulloblastoma was the Group 3/4 (76.2%), followed by the SHH subtype (23.8%), and no WNT subtype was reported (Figure-2). Nuclear β-catenin negativity was observed in all cases, confirming the accuracy of subgroup assignment. Cytoplasmic and nuclear YAP1 expression was consistently noted in SHH tumors, whereas TP53 overexpression was detected in 4 cases (19.0%), primarily associated with large cell/anaplastic histology.

Correlation Between IHC Markers and Histological Patterns

Statistical analysis revealed a significant association between YAP1 expression and the desmoplastic/nodular histological pattern ($p = 0.021$). In contrast, TP53 overexpression did not show a significant correlation with histological subtype ($p = 0.38$), and β-catenin expression was not significantly associated with age group ($p = 0.47$). These findings suggest that YAP1 may serve as a reliable immunohistochemical marker for identifying the SHH subgroup, particularly in desmoplastic/nodular tumors, while TP53 and β-catenin expression patterns reflect subgroup-specific molecular characteristics rather than demographic variables.

FIGURE-2

Illustrates the distribution of molecular subtypes based on immunohistochemical profiles. The predominant subgroup was Group 3/4 (76.2%), followed by the SHH subtype (23.8%), while the WNT subtype was not observed (0%).



DISCUSSION

Medulloblastoma is the most prevalent malignant brain tumour in children and it's now understood that this disease is medically and clinically diverse, meaning that prognosis and potential treatment options must be takend into consideration of the underlying molecular make up of each medulloblastoma tumor type. This study featured a cohort of patients with medulloblastoma whose diagnosis was confirmed by Immunohistochemistry (IHC) using β-Catenin, YAP1 and TP53, and through molecular grouping into the major molecular subtypes. This information provides an alternative option to genomic methods of testing, which may be unfeasible in many locations throughout the world due to financial or resource limitations. This study showed that males had a predominance in this population (M:F = 1.6:1) as seen in other populations experiencing medulloblastoma at this time.[1]

The average age of patients in this cohort was 9.2 years, and this number falls within the expected global peak incidence for paediatric patients diagnosed with medulloblastoma.[2,4] The classic variant of medulloblastoma was the most common histological type identified within the study cohort (76.2% of all medulloblastoma cases). This finding supports other studies which demonstrate that classic was the most prevalent variant in other regions across the globe.[19,20] In addition, both desmoplastic/nodular and large cell/anaplastic histological variations of medulloblastoma add to the existing heterogeneity seen among the medulloblastoma epidemic in this cohort. Molecular classification by immunohistochemistry

showed that the Group 3/4 (76.2%) was common (23.8%), followed by Sonic Hedgehog (SHH) molecular grouping. While, previous reports indicating that SHH was the most commonly identified molecular grouping for children diagnosed with medulloblastoma at under 10 years of age, while WNT group was not identified.[1,18]

We identified the four molecular sub-groups of medulloblastoma (Med) using immuno-histo-chemistry. These four sub-groups include Sonic hedgehog (SHH), WNT, and Groups 3 and 4. This technique was shown to be feasible for clinical use and less expensive than molecular techniques when distinguishing between all four groups, as each sub-group was found to have unique histo-pathological relationships.[16,17] Our data adds to the epidemiological understanding of medulloblastoma in Pakistani children, while also providing additional molecular information absent from the previous studies conducted.[18,21]

Our study's limitations are the small sample size and that the sub-groups were determined by immuno-histo-chemistry rather than genomic assays, as suggested in the literature. In addition, there is limited access to more advanced techniques such as NanoString and next-generation sequencing; thus, if access to either of these technologies continues to be limited, immuno-histo-chemistry will remain a useful tool for determining molecular sub-groups.

Our results confirm that immuno-histo-chemical markers accurately classify medulloblastomas into molecular sub-groups, although the novel observations that Groups 3 and 4 tumours predominate followed by SHH, further corroborated the association of YAP1 expression and desmoplastic/nodular histopathology; all of which are also noted in the literature. Collectively, these data substantiate the clinical utility of immuno-histo-chemistry for classifying Meds for prognostic and treatment options. Finally, further studies will improve both the precision and prognostic significance of molecular sub-group classifications through increased sample sizes and the combined use of molecular assays, such as NanoString and next-generation sequencing.[1,2]

CONCLUSION

Immunohistochemistry for β-catenin, YAP1, and TP53 provides a practical and reliable method for molecular subgrouping of medulloblastoma, with Group3/4 being the most frequent subtype in our cohort. YAP1 correlated significantly with desmoplastic/nodular tumors. These findings support the feasibility of IHC-based classification in routine practice, offering valuable prognostic and therapeutic guidance, especially in resource-limited settings. The study also provides important local data on pediatric medulloblastoma subtypes, underscoring the need for larger, molecularly integrated studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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2	**Zonaira Rathore:** Data collection.
3	**Fizza Jahangir:** Data analysis.
4	**Sameen Afzal:** Writing.
5	**Samina Zaman:** Data collection.
6	**Akhtar Sohail Chughtai:** Critical revisions.