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Research Article

Immunohistochemical study of β catenin expression in medulloblastoma and its clinicopathological correlation

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Abstract

Introduction: *Medulloblastoma is the most common of embryonal tumors and most malignant CNS tumor arising in childhood. 4 core molecular subtypes of medulloblastoma are defined named as WNT (Wingless pathway), SHH (Sonic Hedgehog), Group 3 and Group 4.WNT subgroup is best documented and distinguished by nuclear β catenin immunostaining, Catenin β 1 mutations, chromosome 6 loss, alongside its associated favorable prognosis.*

Materials and Methods: *A laboratory based descriptive type of observational study was carried out in our institute during the year 2017-2018. Total 60 specimens of medulloblastoma were taken. Details about clinical history, histopathological examination and immunohistochemistry were taken and analyzed.*

Results: *Medulloblastoma studied showed male predominance with highest prevalence in 1-10 years age group. Classic subtype was most common and most common site was midline. β catenin immmunoexpression was studied in all specimens, it was cytoplasmic positive in 40 cases and negative in 20 cases. Its expression is correlated with various epidemiological variables like age, sex, desmoplasia, apoptosis, anaplasia, necrosis etc., however no significant statistically difference was seen in relation to these variables.*

Conclusion: *Studies aimed at correlating treatment protocols and outcome with these histological and molecular subtypes are warranted to optimize treatment protocols and for prognostication. While we utilized antibody β-catenin to identify the WNT subtype of medulloblastomas, a larger panel of antibodies like GAB-1, YAP-1 and β Catenin are required to better characterize this heterogeneous group of tumors.*

Keywords: *Medulloblastoma, β Catenin, Immunohistochemistry.*

Introduction

Medulloblastoma is the most common of the embryonal tumor and the most malignant CNS tumor arising in childhood. In children it comprises approximately one quarter of all intracranial tumors, occurring mostly during the first decade of life with a peak age of 7 years, with a slight male predilection.[1]

Current classification schemes for medulloblastoma are based primarily on morphology (histopathology) and include variants such as classic, desmoplastic/ nodular, MBEN

(medulloblastoma with extensive modularity), large cell / anaplastic (LC/A) medulloblastoma.

Current mechanisms for clinical prognostication and stratification include clinical factors (age, presence of metastases, and extent of resection) as well as histological sub grouping (classic, desmoplastic, MBEN and large cell/ anaplastic histology). Recent studies have greatly improved the understanding of Medulloblastoma oncogenesis through elucidation of several developmental signaling pathways. These pathways involve cell signaling receptors, intracellular second messengers, transcription factors and gene regulation.

Many authors have combined data from clinical, pathological and molecular analysis to identify 4 to 6 distinct medulloblastoma variants, although current consensus is that there are only 4 core molecular subtypes of medulloblastoma.[2]

The WNT (wingless pathway) and SHH (sonic hedgehog) were named for the signaling pathways thought to play prominent roles in the pathogenesis of that subgroup. Since less is known about the biology of the remaining two subgroups, the consensus was to retain generic names for the present until the underlying biology driving these subgroups was better delineated.[3]

The constitutive activation of developmental signaling pathways plays a key role in medulloblastoma pathogenesis, and pathway components represent the major mutational targets identified in the disease to date.

WNT signaling regulates the amount and localization of β catenin, a protein involved in both cell adhesion and transcription control. Pathway activation results in the stabilization of β catenin and its translocation into the nucleus. Nuclear β- catenin forms bipartite complexes with T- cell factor (TCF) / lymphoid enhancer-binding factor (LEF) factors and induces transcription of C-Myc, cyclin D, and other genes.[4]

The WNT subgroup is best documented, and is distinguished by nuclear β- catenin immunostaining, Catenin (Cadherin-Associated Protein), Beta 1(CTNNB1) mutations and chromosome 6 loss, alongside its associated favorable prognosis. Mutations in components of the canonical WNT signaling pathway have been described in up to 20% of cases.The SHH pathway plays an essential role in normal cerebellar development, is activated by PTCH (protein patched homolog) 1 mutations in around 10% of human primary medulloblastomas and promotes medulloblastoma development in mouse models of the disease.[5]

Importantly, these pathways appear to have therapeutic significance; WNT- active cases are associated with a favorable prognosis (> 90% overall survival).These medulloblastoma molecular subgroups have significant potential to-

1. Improve clinical management, through molecular disease risk stratification strategies and the identification of patients who could benefit from SHH and WNT targeted molecular therapeutics, and
2. Provide a basis for biological investigations to further understand disease molecular pathogenesis and its therapeutic applications.

According to World health organization (WHO) classification of tumors of central nervous system medulloblastomas are classified under embryonal tumors as:[6] Medulloblastoma genetically defined:

- Medulloblastoma WNT activated
- Medulloblastoma SHH activated and TP53 mutant
- Medulloblastoma SHH activated and TP 53 wild type
- Medulloblastoma non WNT/ non SHH
    - Medulloblastoma group 3
    - Medulloblastoma group 4

1. Medulloblastoma histologically defined:
    - Medulloblastoma classic
    - Medulloblastoma, desmoplastic/nodular
    - Medulloblastoma with extensive nodularity
    - Medulloblastoma, large cell / anaplastic
2. Medulloblastoma, Not otherwise specified (NOS)

This study aims at expression of β catenin in medulloblastoma by immunohistochemistry (IHC) and its clinicopathological correlation.

Materials and Methods

A laboratory based descriptive type of observational study was carried out in our institute during the year 2017-2018.After taking approval from institutional ethics committee, total 60 consecutive specimens of medulloblastoma received during study period were included. Clinical data like age, sex, chief complaints, radiological details were collected in a predesigned and semi-structured performa. Histopathological examination of all specimens was done and immunohistochemistry for β catenin was used. Very small tissue and autolyzed tissue specimen were excluded from the study. Data was entered in Microsoft Excel 2010 and analyzed using chi square test of significance. P-value < 0.05 was considered significant.

Observations and Results

During the period of two years from January 2017 till completion of study, a total of 1004 CNS Specimens were received for histopathological examination of which 60 specimens of medulloblastoma were included.

Mean age in our study was15.88 ± 15.83 years. we found that maximum number of subjects (51.7%) belonged to 1-10 years of age group ; we also found 1 case in ≤ 1year of age and 1 case above 60 years. We found medulloblastoma more in males (75%).Most of the patients in our study presented with vomiting, headache followed by difficulty in walking and restlessness and irritability. Diplopia was present in 12 subjects and 10 patients presented with papilloedema. It was found that midline location was seen in 42 subjects (70%) while 18(30%) subjects had their location in cerebellar hemispheres.

We found four histological subtypes of medulloblastoma in our study population. The predominant subtype corresponded to the Classic variant at 60% (36 subjects). Desmoplastic medulloblastomas formed the next major group with 23.33% (14 subjects), followed by the medulloblastoma with extensive modularity at 10% and large cell/ anaplastic variant in 6.67%.

Out of 60 subjects connective tissue desmoplasia was present in 29 subjects (48.33%) while apoptosis was present in all 60 subjects. Apoptosis was graded into focal, diffuse and extensive. It was found to be focal in 29 subjects , diffuse in 24 and extensive in 7 subjects**.** Necrosis was present in 29 subjects and absent in 31 subjects.Anaplasia was seen in 13 subjects (after exclusion of 4 cases of large cell/ anaplastic variant of medulloblastoma) and was absent in 47 subjects (78.4%).

In our study of 60 subjects β catenin immune-expression was assessed by immunohistochemistry. Cytoplasmic β catenin immune-expression was seen in 40 subjects (66.67%) while none of the subject showed nuclear expression. 20 (33.33 %) subjects were negative for both nuclear and cytoplasmic β catenin expression. (Table 1)

We divided our subjects into two groups – one with absent β catenin immuno-expression and other with cytoplasmic β catenin immuno-expression and compared both groups in relation to different clinicopathological variables like age distribution, sex, site of tumor, histological pattern, desmoplasia, apoptosis, necrosis, anaplasia**.** However we found no statistically significant difference in β catenin absent immune-expression and β catenin cytoplasmic expression in relation to these variables.(Table 2)

Table 1: Demographic, clinical and Histopathological characteristic of Medulloblastoma patients

Variable		N (%)
Age (Years)	**Mean ± SD**	15.88 ± 15.83
	Median (range)	10 (0.7 – 65)
Gender	**Male**	45 (75%)
	Female	15 (25%)
Site of tumor	**Midline**	42 (70%)
	Cerebellar Hemispheric	18 (30%)
Histological type	**Classic**	36 (60%)
	Nodular / Desmoplastic	14 (23.33%)
	MBEN	6 (10 %)
	Large / Anaplastic cell	4 (6.67%)
Connective tissue desmoplasia	**Present**	29 (48.33%)
	Absent	31 (51.67%)
Apoptosis	**Focal**	29 (48.33%)
	Diffuse	24 (40%)
	Extensive	7 (11.67%)
Necrosis	**Present**	29 (48.33%)
	Absent	31 (51.67%)
Anaplasia	**Present**	13 (21.6%)
	Absent	47 (78.4%)
β Catenin	**Nuclear**	0 (%)
	Cytoplasmic	40 (66.67%)
	Negative	20 (33.33%)

Table 2: Factor associated with pattern of β Catenin expression among Medulloblastoma patients

Variable	Sub group	β Catenin		Total (N= 60)	Chi square P value
		Negative (N=20)	Cytoplasmic (N=40)		
Age group	**Infant (≤ 1 years)**	0 (0%)	1 (2.5%)	1	χ^2= 1.587 P = 0.452
	Children (1 – 15 years)	15 (75%)	24 (60%)	39	
	Adults (>15 years)	5 (25%)	15 (47.5%)	20	
Gender	**Female**	6 (30%)	9 (22.5%)	15	χ^2 = 0.100 P = 0.752
	Male	14 (70%)	31 (77.5%)	45	
Site of tumor	**Midline**	14 (70%)	28 (70%)	42	χ^2 = 0.089 P = 0.765
	Hemispheric	6 (30%)	12 (30%)	18	
Histological type	**Classic**	12 (60%)	24 (60%)	36	χ^2 = 0.150 P = 0.928
	Nodular / Desmoplastic	4 (20%)	10 (25%)	14	
	MBEN	3 (15 %)	3 (7.5%)	6	
	Large / Anaplastic cell	1 (5%)	3 (7.5%)	4	
Connective tissue desmoplasia	**Present**	12(60%)	17 (42.5%)	29	χ^2 = 0.082 P = 0.774
	Absent	8 (40%)	23 (57.5%)	31	
Apoptosis	**Focal**	10 (50%)	19 (47.5%)	29	χ^2 = 0.490 P = 0.783
	Diffuse	7 (35%)	17 (42.5%)	24	
	Extensive	3 (15%)	4 (10%)	7	
Necrosis	**Present**	10 (50%)	19 (47.5%)	29	χ^2 = 0.008 P = 0.927
	Absent	10 (50%)	21 (22.5%)	31	
Anaplasia	**Present**	4 (20%)	9 (22.5%)	13	χ^2 = 0.027 P = 0.869
	Absent	16 (80%)	31 (77.5%)	47	

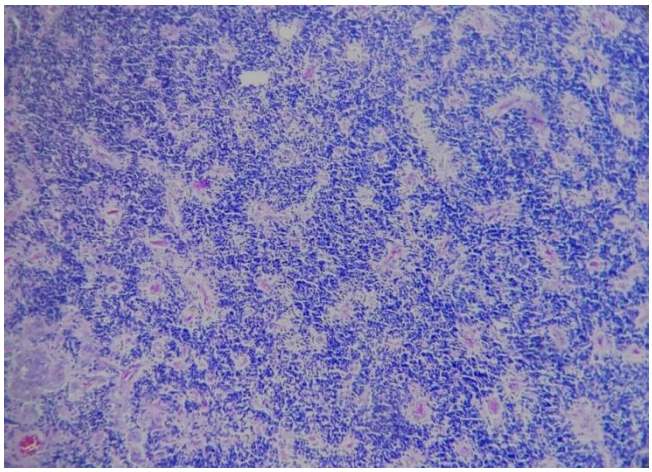

Figure 1: H & E stained section showing classic variant of medulloblastoma with areas of Homer Wright rosettes (100x).

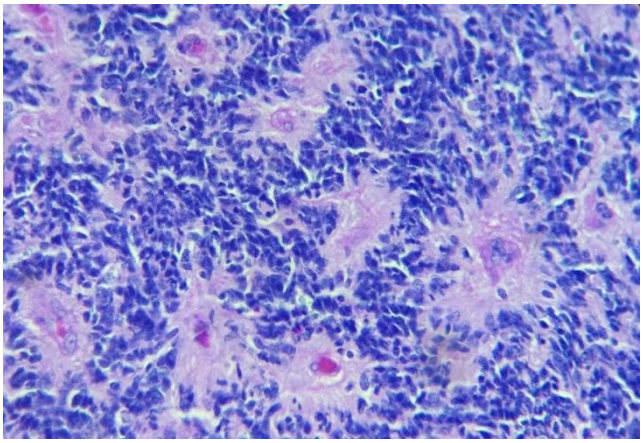

Figure 2: H & E stained section showing classic variant of medulloblastoma with areas of Homer Wright rosettes (400x)

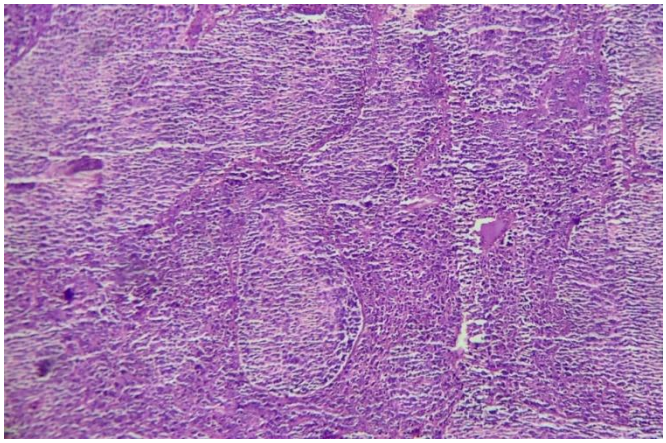

Figure 3: H & E stained section showing desmoplastic/ nodular variant with pale nodules surrounded by intervening hypercellular internodular areas (400x)

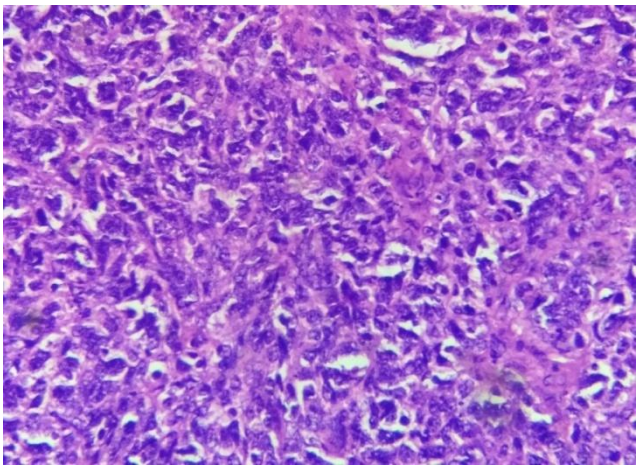

Figure 4: H & E stained section showing large cell / anaplastic variant with large polygonal cells, nuclear pleomorphism and prominent nucleoli (400x)

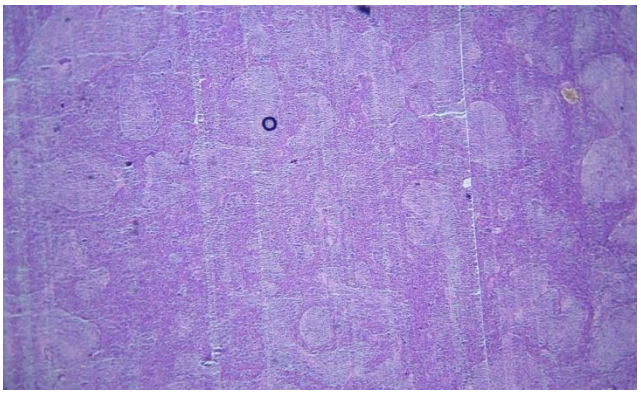

Figure 5: H & E stained section showing MBEN with an expanded lobular architecture characterized by large elongated reticulin free zones (100x)

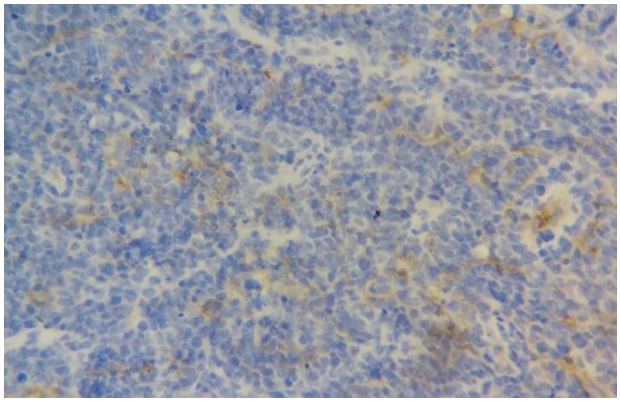

Figure 6: Negative β catenin immunostaining in a case of medulloblastoma (400x)

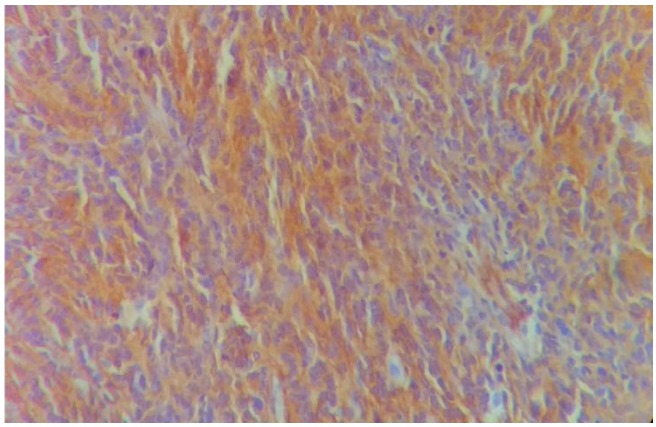

Figure 7: Cytoplasmic β catenin immunostaining in a case of medulloblastoma (400x)

Discussion

Medulloblastoma is a complex disease, characterized by described genetic changes that have facilitated a molecular classification of these tumours. The molecular classification of medulloblastomas is aimed at enabling the prediction of the pathogenesis and biological behavior.

Historically, tumour classification has been based on histological classification alone, and management based on clinical characteristics that stratify patients into low or standard risk groups. However, studies have shown varied responses to treatment, and varied outcomes have been described for the same histologic subtype, with patients who are potentially curable with less aggressive management strategies suffering significant treatment related morbidity, thus highlighting the need a molecular classification. Patient management thus far has been based on standard therapy protocols There is evidence of unique molecular pathogenetic pathways for medulloblastoma. However, there is a need for accessible techniques that can identify surrogate markers for these molecular pathways that are potentially usable in routine diagnostic pathology, specifically immunohistochemistry and FISH[7].

Nearly all of the WNT medulloblastomas studied have classic histology. WNT medulloblastomas are frequently described as having CTNNB1 mutations, nuclear immunohistochemical staining for β-catenin and monosomy six. Medulloblastomas with large cell / anaplastic histology have also been reported in the WNT subgroup, although they appear to maintain the excellent prognosis associated with the WNT subgroup. Overall medulloblastoma is more common in males, however the gender ratio for WNT medulloblastoma is about 1:1 male: female. WNT medulloblastomas can occur at all ages, but are uncommon in infants. As most patients with WNT medulloblastoma have a good prognosis, it is possible that they are being over treated with current therapies which are quite morbid and there is an active discussion of a clinical trial of therapy de-escalation in this patient population.[3]

The present study was carried out with the aim to assess the pattern of β-catenin expression in medulloblastoma by immunohistochemistry and its clinico-pathological correlation.

In the present study mean age was slightly higher with significantly higher incidence in males as compared to previous studies [8,9,10]. This difference in age and gender distribution can be explained due to different demographic and clinical characteristics of the patients.

The majority of the cases were midline in location with most common histological type being classical variant followed by desmoplastic / nodular, medulloblastoma with extensive modularity and large cell anaplastic variant. These findings were in concordance with other studies [8,9,11,12].

John Pizem et al in their study that apoptotic rate was higher in medulloblastoma with CSF disseminaton and in desmoplastic medulloblastoma. However it has been found to have no prognostic value[13]. Apoptosis was seen in all cases of medulloblastoma in the present study with 43 % cases showing focal apoptosis. In our study necrosis was present in 48.33 % cases and absent in 51.67 % cases. These results are in favor with other studies[9,14] but these results contrast with study done by Katshushi Taomoto et al[15] (1987) which had necrosis in 70.8 % cases and absent in 29.2 % cases. Demoplasia was present in 48.3 % cases while absent in 51.7 % cases.

Similar results were seen by other studies[9,16]. In our study we found that 67% patients showed only cytoplasmic beta catenin positivity. Rest were negative for beta catenin expression. None of the case showed nuclear positivity for beta catenin. Similar results were seen by Rehab M. Samaka[9]et al with no case showing nuclear localization of beta catenin. Another study by Satoshi Utsuki et al[17] studied 13 medulloblastomas, they found that no nuclei of the medulloblastomas were stained for beta catenin. It was present in cytoplasm (cell-cell borders in 12 of 13 medulloblastomas). This suggests absence of role of WNT signaling pathway. However for definite confirmation CTNNB1 mutation and presence of monosomy six should be evaluated. Presence of cytoplasmic β- Catenin positivity also indicates that other signaling pathways may play a major role in pathogenesis of our cases. It may be seen in cases of SHH and non - WNT/ non- SHH. However other markers like Growth factor receptor-bound protein 2 associated-binding protein 1(GAB1) and Yes associated protein (YAP 1) are needed to confirm it.

We divided our subjects into two groups – one with absent β catenin immuno-expression and other with cytoplasmic β catenin immuno-expression and compared both groups in relation to different clinicopathological variables like age distribution, sex, site of tumor, histological pattern, desmoplasia, apoptosis, necrosis, anaplasia**.** However we found no statistically significant difference in β catenin absent immune-expression and β catenin cytoplasmic expression in relation to these variables. These results are in favor with a study by Rehab M. Samaka et al[9], they also found no statistically significant difference between the two groups in relation to all these variables.

Conclusion

Medulloblastoma is a common malignant embryonal childhood tumor located in the cerebellum; Molecular sub grouping has allowed for categorization of medulloblastomas in ways that have prognostic and therapeutic significance. The present study represents the first step in characterization of the cohort of cases seen in our institution. Apart from expanding the panel of markers to completely characterize this cohort, future studies will require incorporation of more detailed genomic platforms to segregate these tumors in appropriate subtypes. Studies aimed at correlating treatment protocols and outcome with these histological and molecular subtypes are warranted to optimize treatment protocols and for prognostication. While we utilized antibody β-catenin to identify the WNT subtype of medulloblastomas, a larger panel of antibodies is required to better characterize this heterogeneous group of tumors.

A diagnostic immunohistochemical method has been defined that can distinguish between WNT activated, SHH activated and non WNT/ non SHH tumours using formalin- fixed paraffin -embedded material. A panel of 3 antibodies have been suggested-GAB-1, YAP-1 and β Catenin. GAB-1 and YAP-1 are the immunohistochemical markers indicating SHH activation. The anti-GAB1 antibody labelled only tumours with an SHH activated profile, whereas the anti-YAP1 antibody labelled tumour cells in both WNT activated and SHH activated medulloblastomas. With a panel of 3 antibodies (to beta catenin, GAB1 and YAP1), non WNT/non SHH tumors show cytoplasmic (but not nuclear) beta catenin immunoreactivity, and the tumor cells are immunonegative for GAB 1 and YAP1.

Although histological subtyping of medulloblastomas has not been entirely supplanted, it is quite clear that histology alone is irrelevant in isolation and that identification of molecular sub groups is of paramount importance clinically. The challenge we face in the Indian setting, is to develop simple inexpensive techniques for sub- typing that can be adopted universally. Inter-center cooperation and setting up of core facilities for testing may be the way forward.

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