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ORIGINAL RESEARCH

Histopathological assessment and expression of beta catenin, N cadherin and calbindin in medulloblastoma subtypes

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ABSTRACT

AIM: To asses and compare the histopathological parameters and biological markers like β-catenin, N- cadherinand Calbindin in medulloblastoma (MB) subtypes.

MATERIAL and METHODS: This retrospective comparative study includes 54 formalin fixed paraffin embedded tumor tissue samplesbetween January 2003 and December 2010. MB was first examined using standard histological preparations.Subsequently, immunohistochemistry was performed using antibody against β- Catenin, N-cadherin and Calbindin.

RESULTS: Themean age in the pediatric age group was 10.7 years and 33 years in adult witha male preponderance.Majority of classic MB were of midline vermian origin (74%) in contrast to the lateral location of desmoplastic MB (69%) (P=0.001).Most common histological subtype was classic (61%). Apoptosis was more commonly present in desmoplastic MB(43%).24% of MB showed the presence of necrosis,mostly in MB with extensive nodularity subtype. Perivascular pseudo-rosette and Homer-Wright rosette formation was observed in 65% of MB as in all age group.59% showed moderate anaplasia followed by mild (30%). All four anaplastic subtypes showed severe anaplasia.β-catenin immunoreactivity was observed in 17 (31%) of the 54 MB. Of the 17 positive cases, 12 (71%) were of classic phenotype.Expression of N-Cadherin (strong nuclear as well as cytoplasmic) was observed in 42 (78%).Only 6 cases showed focal cytoplasmic positivity for calbindin.

DISCUSSION- 24% of medulloblastomas showed the presence of necrosis which is in harmony with previous studies. Previous studies showed that moderate and severe anaplasia are associated with recurrence and metastasis. Focal as well as diffuse cytoplasmic β-catenin immunoreactivity was seen in 17 of the 54 samples. Previous findings propose that accumulation of nuclear β-catenin may possibly be a marker of favorable outcome in MB.

CONCLUSION: This study found that the degree of anaplasia in medulloblastoma subcategories plays an important role in prognostication. Thenuclear accumulation of β-catenin and calbindin an important prognostic parameter was observed in one case.A larger study with adequate clinical follow up is requiredfor consequential association between these biomarkers

Key words: Medulloblastoma, N-Cadherin, β-catenin, calbindin

ABBREVIATIONS:MB: Medulloblastoma, **CNS:** Central nervous system, **EMT:** Epithelial Mesenchymal Transition

INTRODUCTION:

Medulloblastoma (MB) an embryonal tumor of the cerebellum is the most frequent childhood malignant brain tumor. Globally this highly aggressive tumor accounts for about 20% of all central nervous system (CNS) tumors.[1]. As reported earlier 5year survival rates was less than 70% and 10 year survival rates was between 30 to 50%, [2, 3]suggesting the presence of subgroups with different response to radio/chemotherapy. But later significant advances in treatmenthas ledfive-year overall survival rate to 70–90% [4]. Several prognostic and predictive factors have been proposed in medulloblastomas in the context of therapeutic trials. Novel therapies for aggressive disease and the accurate identification of risk groups would probably facilitate the targeted use of adjuvant therapies. Different biological markers including Ca^{2+}binding proteins are involved in the regulation of cell proliferation, programmed cell death, invasion and metastasis[5, 6]. Hence we included $Ca2^{+}$binding proteinsβ-catenin, (N- cadherin) and Calbindin in our study.

β-catenin a lining protein is identified to undergo nuclear localization and mutation in MB.It is an essential structural element of cell adhesion junctions, β-catenin demonstrate considerable improvementin the survival rate of the patient as the mutations occurred are associated with positive nucleus immune phenotyping and these findings proposes that accumulation of nuclearb-catenin possibly will be a marker of favorableoutcome in MB.[7] The post-translational alterations(phosphorylation, glycosylation,ubiquitylationandacetylation) can influence the functional output of β-catenin.[8] While N- cadherin a cell surface glycoprotein is involved in $Ca2^{+}$ binding cell – cell adhesion which is essential for maintenance of normal tissue organization and are also plays an imperative role during morphogenesis and histogenesis.[9]. Theses Cadherins binds to β-catenin. Calbindin expression was reportedin a subset of medulloblastomaspresenting poorly differentiated cells while inmedulloblastomas with desmoplastic characteristics it was absent.[10] Calbindin -positive medulloblastomas were reported to represent a subtypes of aggressive tumors more often seen in youngerpatients.[11]Hence, the present study aims at assessment of histopathological parameters and biological markers like β-catenin, cell surface glycoprotein (N- cadherin) and Calbindin in medulloblastoma subtypes.

MATERIAL and METHODS

This retrospective comparative study was conducted at theDepartment ofRadiotherapy and Pathology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. The total 65 cases retrieved from the department of pathology between January 2003 and December 2010.Out of these, 54 formalin fixed paraffin embedded tumor tissue samples were evaluable and only 24 cases were followed up from the department ofRadiotherapy.

Immunohistochemistry

MB was first examined using standard histological preparations (hematoxylin and eosin, reticulin). Subsequently, immunohistochemistry was performed using antibody against β-Catenin, N-cadherin and Calbindin. The paraffin embedded sections were collected on poly-L-lysine coated slides and de-paraffinized in xylene, gradually rehydrated in absolute alcohol, 70% alcohol, 50% alcohol and tris buffered saline (TBS,pH7.4). For β-catenin immunohistochemistryantigen retrieval was performed by treating sections in pressure cooker in citrate buffer (pH 6.2),for N-cadherinbytreating the section with proteinase K andfor

Calbindin antibody microwave antigen retrieved in citrate buffer (pH 6.2). The endogenous peroxidase activity was blocked by treating the sections with 3% H2O2 in 97% methanol for 20 minutes in dark followed by thorough wash in TBS. The nonspecific binding sites were blocked by using protein block. The primary antibody was diluted in 1:200 for β- catenin, 1:600 for N
-cadherin and 1:300 for Calbindin respectively (source- sigma). After incubation with primary antibody at room temperature for 3 hours, the slides were washed with TBS for 3 times (15 minutes each) and subsequently incubated with secondary antibody (biotinylated) and HRP conjugated streptavidin (30min each). Then the sections were washed (15x3 min) in TBS and color reactions were developed by DAB (di-amino-benzidine) and H2O2 as substrate chromogen (source- DAKO). The sections were then lightly counterstained with hematoxylin. With each batch of stain, positive controls were antral biopsies for β-catenin, infiltrating duct carcinomas of breast for N-cadherin and normal cerebellar tissue for Calbindin. non-immune serum used as negative control.β-catenin positivity- cytoplasmic or combined cytoplasmic/nuclear staining. N-cadherin positivity- nuclear and cytoplasmic. Calbindin positivity- cytoplasmic.

Expression analysis

For β-catenin, nuclear positivity in at least 10% of the tumor cells were considered as positive.However tumor cells with cytoplasmic positivity was also counted separately. Similarly a minimum of 25% of cytoplasmic positivity in tumor cell population was considered as positive for Calbindin and N-Cadherin.

Statistical Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) Version 17.0 Data has been represented as frequency and percentages. Measures of association were calculated using Chi-square test. The confidence level of the study was kept at 95% and hence a “p” value less than 0.05 indicated a statistically significant association.

RESULTS

Table-1 showed the distribution of Sex and location of tumorwith reference to age, among total 54 study participants, 40 (74%) were in the pediatric age group (up to 16years) and 14 (26%) were in the adult age group (beyond 16years). Mean age was 10.7 years and 33 years in the pediatric age group and adult age group respectively witha male preponderance (78%).Vermis was the most common site of involvement (76%).

As per Table-2most common histological subtype was classic MB found in both pediatric (67%) and adult (43%) followed bydesmoplastic MB (present in 20% pediatric and 43% adult population). Cases of MB with extensive nodularity, were seen only in pediatric population (8%). Majority of classic MB were of midline vermis origin (74%) in contrast to the lateral location of desmoplastic MB (69%).Apoptosis was more commonly present in desmoplastic MB (43%) as compared to classic MB (12%).

Table-3 demonstrates that among the study population, 24% of MB showed the presence of necrosis and most commonly seen in MB with extensive nodularity subtype. Perivascular pseudo-rosette and Homer-Wright rosette formation was observed in 65% (n=35) of MB in all age group.Most of the MB in this study showed moderate anaplasia (59%) while all 4(100%) anaplastic subtypes showed severe anaplasia.

As perTable-4, β-catenin immune-reactivity was observed in 17 (31%) of the total 54 MB cases (Fig.5&6). Of the 17 positive cases, 12 were of classic phenotype, 4 haddesmoplastic morphology and 1case was labeled as desmoplastic MB with extensive nodularity. (Fig 1,2 &3) Expression of N-Cadherin (strong nuclear as well as cytoplasmic) was observed in all

subtypes of MB. 42(78%) out of 54 MB cases were showing expression. Of the 42 positive cases, 24 were of classic morphology, 12 haddesmoplastic morphology, 2cases were labeled as desmoplastic MB with extensive nodularity while 4 were of anaplastic subtype (Fig 2,3 & 4) Majority of the MBs (89%) were negative for Calbindin. Only 6 cases showed focal cytoplasmic positivity which was confined to the areas having neuronal maturation. Some amount of nuclear positivity was also observed. (Fig 9 & 10).

Table1. Distribution of Sex and location of tumor with reference to Age

Age in years			Sex			location of tumor		
	No of subjects	**Mean±SD**	**Male**	**Female**	**Total**	**Vermis**	**Hemisphere**	**Total**
Up to 16	40 (74%)	10.7±3.92	30 (75%)	10 (25%)	40 (100%)	33 (82%)	7 (18%)	40 (100%)
Beyond 16	14 (26%)	33±10.95	12 (86%)	2 (14%)	14 (100%)	8 (57%)	6 (43%)	14 (100%)
Total	54 (100%)	16.7±11.77	42 (78%)	12 (22%)	54 (100%)	41 (76%)	13 (24%)	54 (100%)
Statistical analysis Chi-Square Test			P=0.407			P=0.05		

Table 2. Histological distribution with reference to Age, location of tumor and Apoptosis

Histological Subtype	**Age**		**location of tumor**		**Apoptosis**		**Total**
	Up to 16	Beyond 16	Vermis	Hemisp-here	Present	Absent	
Classic medulloblastoma	27 (67%)	6 (43%)	30 (74%)	3 (23%)	4 (12%)	29 (88%)	33 (100%)
Desmoplasticmedullob lastoma	8 (20%)	6 (43%)	5 (12%)	9 (69%)	6 (43%)	8 (54%)	14 (100%)
Medulloblastoma with extensive nodularity	3 (8%)	0 (0%)	3 (7%)	0 (0%)	0 (0%)	3 (100%)	3 (100%)
Anaplastic/large cell medulloblastoma	2 (5%)	2 (14%)	3 (7%)	1 (8%)	2 (50%)	2 (50%)	4 (100%)
Total	40 (100%)	14 (100%)	41 (100%)	13 (100%)	12 (22%)	42 (78%)	54 (100%)
Statistical analysis Chi-Square Test	P=0.146		P=0.001		P=0.45		

Table 3. Histological distribution with reference to Necrosis, Rosette formation and Anaplasia

Histological Subtype	**Necrosis**		**Rosette formation**		**Anaplasia**		
	Present	Absent	Present	Absent	Mild	Moderate	Severe
Classic medulloblastoma	10 (30%)	23 (88%)	26 (79%)	7 (21%)	12 (36%)	20 (60%)	1 (4%)
Desmoplasticmedullob lastoma	1 (7%)	13 (93%)	6 (43%)	8 (57%)	2 (14%)	11 (79%)	1 (7%)
Medulloblastoma with extensive nodularity	1 (33%)	2 (67%)	1 (33%)	2 (67%)	2 (67%)	1 (33%)	0 (0%)
Anaplastic/large cell medulloblastoma	1 (25%)	3 (75%)	2 (50%)	2 (50%)	0 (0%)	0 (0%)	4 (100%)
Total	13 (24%)	41 (76%)	35 (65%)	19 (35%)	16 (30%)	32 (59%)	6 (11%)
Statistical analysis Chi-Square Test	P=0.386		P=0.058		P=0.021		

Table 4.Medulloblastoma subtypes & expression of β catenin, N-Cadherin and Calbindin

Histological subtype	**β-Cantenin**		**N-Cadherin**		**Calbindin**		**Total**
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	
Classic medulloblastoma	12 (36%)	21 (64%)	24 (73%)	9 (27%)	2 (6%)	31 (94%)	33 (100%)
Desmoplasticmedulloblast	4	10	12	2	3	11	14

oma	(29%)	(71%)	(86%)	(14%)	(21%)	(79%)	(100%)
Medulloblastoma with extensive nodularity	1 (33%)	2 (67%)	2 (67%)	21 (33%)	1 (33%)	2 (67%)	3 (100%)
Anaplastic medulloblastoma	0 (0%)	4 (100%)	4 (100%)	0 (0%)	0 (0%)	4 (100%)	4 (100%)
Total	17 (31%)	37 (69%)	42 (78%)	12 (22%)	6 (11%)	48 (89%)	54 (100%)

p=0.520(Chi-Square Test)

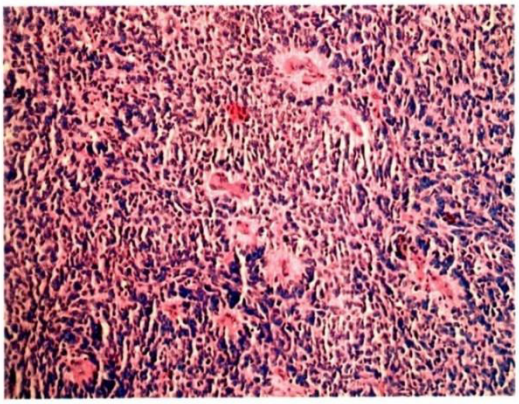

Figure 1. Classic medulloblastoma displaying densely packed small round cells (H&E, 20x)

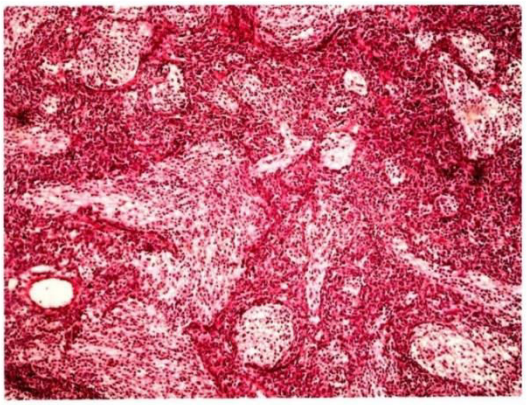

Figure 2. Desmoplastic medulloblastoma with pale nodules (H&E, 10x)

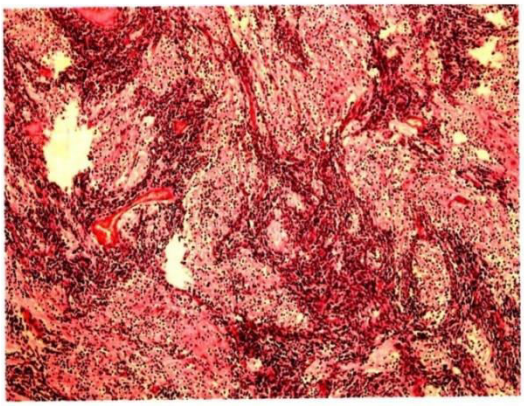

Figure 3. Medulloblastoma with extensive nodularity (H&E, 20x)

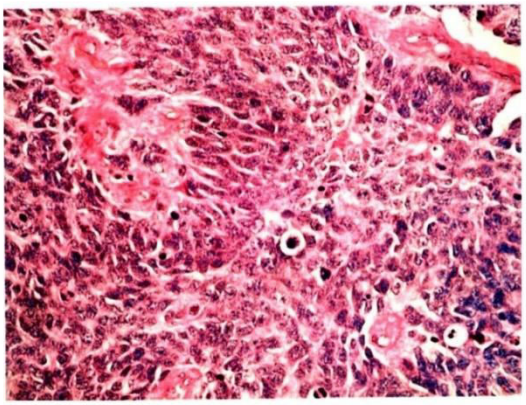

Figure 4. Anaplastic/large cell medulloblastoma displaying endothelial proliferation (H&E, 40x)

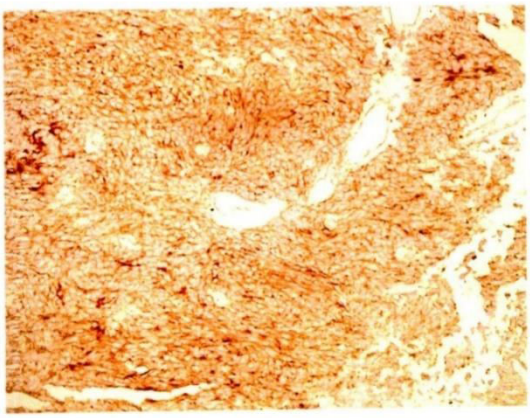

Figure 5. Predominantly membranous β-catenin positivity in classic medulloblastoma (Immunostain, 20x)

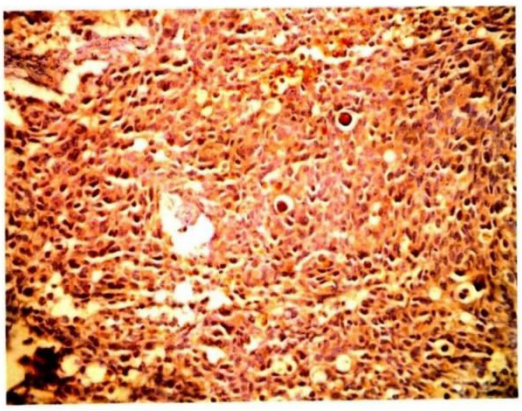

Figure 6. Cytoplasmic β-catenin positivity in classic medulloblastoma (Immunostain, 40x)

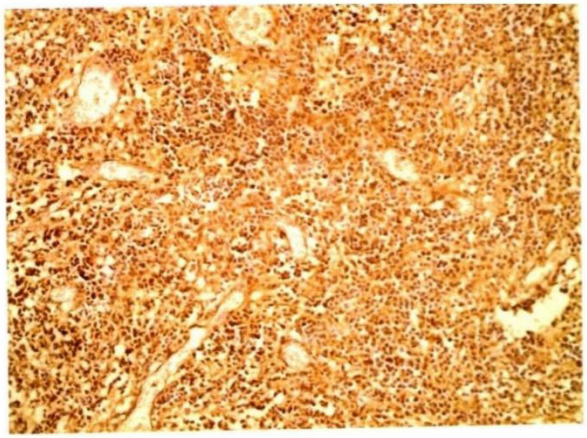

Figure 7.Diffuse nuclear as well as cytoplasmic positivity of N-cadherin (Immunostain, 20x)

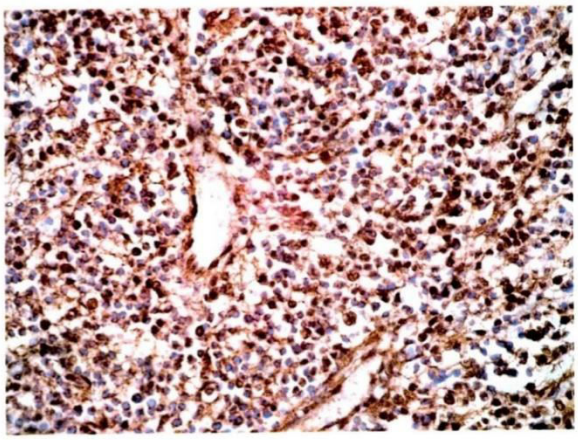

Figure 8. Diffuse nuclear positivity of N-cadherin (Immunostain, 40x)

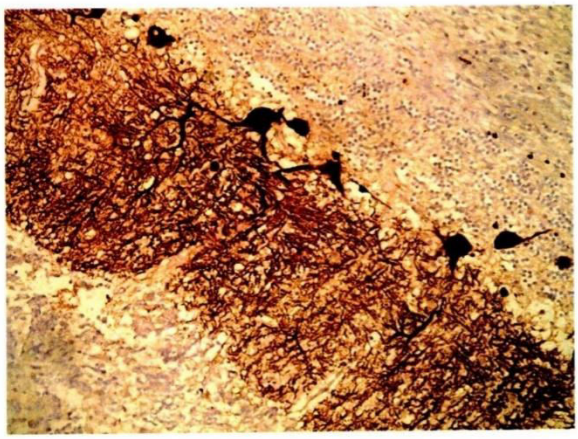

Figure 9. Calbindin positivity in nuclei as well as axons of Purkinje cells of cerebellum with adjacent tumor cells on lower left which are negative (Immunostain, 20x)

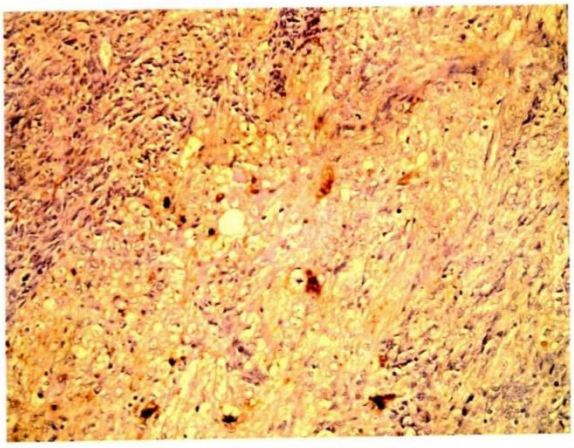

Figure 10. Focal nuclear as well as cytomplasmic calbindin positivity in areas of neurocytic differentiation in classic medulloblastoma (Immunostain, 40x)

DISCUSSION

MB is a highly malignant embryonal neuroectodermal tumor of the cerebellum in pediatric population accounting for approximately 10% of all pediatric cancer-related deaths.[12] In our study the majority (64%) of cases werebelow 16 years with mean age of 10.7 years which was comparable with earlier findings where the patients diagnosed were below 15 years of age; however up to 20% of patients with MB are diagnosed in late adolescence or early adulthood with a peak incidence in the range of 17-34 years[13-15]

Prognosis of MB by histopathological sub grouping depends on the detectionof anaplastic/highly aggressive large cellmorphology or desmoplastic/extensivenodular phenotype in infants [16, 17]. A number of histopathological parameters have been identified which may allow more accurate management of medulloblastoma patients [18]. In this study a detailed histopathological review of medulloblastoma using various morphological variables was carried out.Majority of childhood medulloblastomas in our series had classic histology (n=27) and 74% were located in vermis which is similar to the previous study done bySarkaret al [19]. The nodular/desmoplastic variant has been reported to occur more frequently among adult patients with a preferential location in one of the cerebellarhemispheres[20]. In the present study, classic and nodular/desmoplastic variants

were seen in equal proportion in adults probably due to lesser number of adult cases in the study. Similar to previous studies laterally located tumors were more frequently of desmoplastic morphology (69%). Though WHO classification 2016 has given no recommendationfor the utilization of precise test schemes ortechniques, but emphasized on the execution of molecular markers forclassification that might be probable in every day diagnosticpractice in the majority of laboratories. [21] A recent review [22] focused on the progression of thebiology and genomics of MB and that led to considerable modification in the pathologic identification and classification of MB. They specifically emphasized on recentpractices of neuropathologyfor the histology, pathological identificationand classification of MB.

Apoptosis is a major contributor in cell loss in meduloblastomas [23]. Apoptotic indices generally parallel mitotic indices with the exception of pale nodules of desmoplastic medulloblastoma which have high apoptotic indexbut low growth fraction [24]. In the present study nodular/ desmoplastic medulloblastomas showed a statistically significant extensive intra-nodular apoptosis compared to classic variant. Giangaspero et al found significantly shorter relapse free interval in medulloblastoma patients with severe anaplasia and extensive apoptosis [25].

Necrosis is often associated with rapid tumor proliferation and is a negative prognostic indicator in many central nervoustumor including medulloblastoma[26,27] In our study only 24% of medulloblastomas showed the presence of necrosis which is in harmony with previous studies.Endothelial proliferation was observed in 55% of cases of pediatric and 50% cases of adult medulloblastoma and was statically significant. Maire JP et al found presence of endothelial proliferation is a negative prognostic indicator in medulloblastoma [27]. However other studies showed these feature are not predictive of survival [28, 29].

In our study perivascular pseudo rosette and Homer-Wright rosette was seen in the majority of medulloblastomas (65%).The significance of rosettes is not clear, though it indicates tumor differentiation and helpful in the histologic diagnosis of medulloblastoma[30].Most of the medulloblastomas in this study showed moderate anaplasia (59%) while all four anaplastic subtypes showed severe anaplasia.).Previous studies showed that moderate anaplasia and severe anaplasia are associated with recurrence and metastasis and plays important role in pathologic grading of medulloblastomas[16,31].

We also looked for the surrogate marker of the Wntsignalling pathway like β-catenin, a protein involved in cell adhesion and transcription during the development of cerebellum. Focal as well as diffuse cytoplasmic β-catenin immunoreactivity was seen in 17 of the 54 samples we tested

Several studies have revealed that β-catenin mutations are correlated with positive nucleus immune-phenotyping, with considerable improvement in the survival rate of the patient. Previous findings propose that accumulation of nuclearβ-catenin may possibly be a marker of favorableoutcome in MB, while a recent study on MB cell lines has reported that activation of the Wntsignaling pathway bytransitory expression of β -catenin induced the expressionof its target genes and decreased the number ofMBcell colonies. Consequently, Wnt activation might correspond to anindicator of good prognosis. [7, 32]. Pathway activation has also been associated with better clinical outcome. Activation of Wnt pathway is mediated through the stabilization and nuclear accumulation of β-catenin, and is associated with mutation of its corresponding gene CTNNB1 in majority of pediatriccases [11,33]. However, despite this evidence,the importance of Wnt pathway activation in MB initiation remains to be determined. Also, since manypatients still have long-term side effects related totherapy [34-36], these studies could bring newinsights into MB treatment. In contrast 42 of the 54 medulloblastomas were immunoreactive for N-Cadherin in the tumor cell nuclei as well as in cytoplasm. There was no significant difference in N-Cadherin expression and histological

subtypes. High levels of N-. Utsuki et al observed that the N-Cadherin positive medulloblastomas are more aggressive in terms of dissemination. E- and N-cadherin, as representative EMT markers, have limited prognostic value in glioma. Nonetheless, the EMT process in gliomas may be compounded by enhanced N-cadherin expression supported by unfavorable prognostic outcomes. [37] A very recent review has emphasized on undetermined expression of cadherin in CNS tumors, specifically in gliomas and found that in glioblastoma theswitching procedure of cadherin remains indefinable and in the progression of glioma the cadherin expression involvement is yet uncertain. [38]

Studies on Calbindin and its role in medulloblastoma progression are sparse.In the present study, calbindin expression was observed in 6 cases only of which 4 were in the pediatric age group. Katsetos et al have found calbindin-D28k expression in a subset of medulloblastomaswith poorly differentiated cells but absent in desmoplastic medulloblastomas. [10] Pelc et al observed calbindin positivity almost exclusively in pediatric population with poor prognosis and high risk of tumor recurrence. They also found that in metastatic tumors, the absence of calbindin immunoreactivity correlates with good prognosis [11]. Although calbindin expression is not much studied recently in medulloblastoma specifically but in a recent study on Osteosarcoma(a most common type of primary malignant bone tumor) calbindin1 was found to contribute in shielding osteosarcoma cells from apoptosis and offers a probable novel target for gene therapy to cure patients with this malignancy. [39]

CONCLUSION

This study found that the degree of anaplasia in medulloblastoma subcategories plays an important role in prognostication. The significance of N-Cadherin expression in medulloblastoma subtypes is unclear while nuclear accumulation of β-catenin, an important prognostic parameter was observed in one case and almost same findings were observed with expression of calbindin also.A larger study with adequate clinical follow up is requiredfor consequential association between these biomarkers.

REFERENCES

1. Dhall G: Medulloblastoma. J Child Neurol 24: 1418–1430, 2009
2. Giordana MT, Schiffer P, Schiffer D: Prognostic factors in medulloblastoma. Childs Nerv Syst.14(6):256-62, 1998
3. Whelan HT, Krouwer HG, Schmidt MH, Reichert KW, Kovnar EH: Current therapy and new perspectives in the treatment of medulloblastoma. Pediatr Neurol. 18(2):103-15, 1998
4. Gajjar A, Chintagumpala M, Ashley D, Kellie S, Kun LE, Merchant TE et al: Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue in children with newly diagnosed medulloblastoma (St Jude Medulloblastoma-96): long-term results from a prospective, multicentre trial. Lancet Oncol 7: 813–820, 2006
5. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 1:11-21, 2000;
6. Robinson GW, Rudneva VA, Buchhalter I, Billups CA, Waszak SM, Smith KS, Bowers DC, Bendel A, Fisher PG, Partap S, Crawford JR, Hassall T, Indelicato DJ, Boop F, Klimo P, Sabin ND, Patay Z, Merchant TE, Stewart CF, Orr BA, Korbel JO, Jones DTW, Sharma T, Lichter P, Kool M, Korshunov A, Pfister SM, Gilbertson RJ, Sanders RP, Onar-Thomas A, Ellison DW, Gajjar A, Northcott PA : Risk-adapted therapy for young children with medulloblastoma (SJYC07): therapeutic and molecular outcomes from a multicentre, phase 2 trial. Lancet Oncol 19(6):768-784, 2018
7. Ellison DW, Onilude OE, Lindsey JC, Lusher ME, Weston CL, Taylor RE, Pearson AD, Clifford SC :beta-Catenin status predicts a favorable outcome in childhood

medulloblastoma: the United Kingdom children's cancer study group brain tumour committee. J Clin Oncol 23:7951–7957 2005

8. Valenta T, Hausmann G, Basler K: The many faces and functions of beta-catenin. EMBO J 31:2714- 2736, 2012
9. Takeichi M: The Cadherins: cell cell adhesion molecules controlling animal morphogenesis. Development 102:639-655, 1988
10. Katsetos CD, Herman MM, Krishna L, Vender JR, Vinores SA, Agamanolis DP, Schiffer D, Burger PC, Urich H: Calbindin-D28k in subsets of medulloblastomas and in the human medulloblastoma cell line D283 Med. Arch Pathol Lab Med. 119(8):734-43, 1995
11. Pelc K, Vincent S, Ruchoux MM, Kiss R, Pochet R, Sariban E, Decaestecker C, Heizmann CW: Calbindin-d(28k): a marker of recurrence for medulloblastomas. Cancer 95(2):410-19, 2002
12. Pizer BL, Clifford SC : The potential impact of tumour biology on improved clinical practice for medulloblastoma: progress towards biologically driven clinical trials. Br J Neurosurg. 23:36-375, 2009
13. Roberts RO, Lynch CF, Jones MP, Hart MN : Medulloblastoma: a population-based study of 532 cases. J NeuropatholExpNeurol 50(2):134-44, 1991
14. Hubbard JL, Scheithauer BW, Kispert DB, Carpenter SM, Wick MR, Laws ER Jr: Adult cerebellar medulloblastomas: the pathological, radiographic, and clinical disease spectrum. J Neurosurg70(4):536-44, 1989
15. Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, Troost D, Meeteren NS, Caron HN, Cloos J, Mrsić A, Ylstra B, Grajkowska W, Hartmann W, Pietsch T, Ellison D, Clifford SC, Versteeg R: Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. PLoS One. 3(8):e3088, 2008
16. Eberhart CG, Kepner JL, Goldthwaite PT, Kun LE, Duffner PK, Friedman HS, et al. :Histopathologic grading of medulloblastomas: a Pediatric Oncology Group study. Cancer 94:552-560, 2002
17. Rutkowski S, Bode U, Deinlein F, Ottensmeier H, Warmuth-Metz M, Soerensen N, et al. : Treatment of early childhood medulloblastoma by postoperative chemotherapy alone. N Engl J Med. 352:978-986, 2005
18. Eid AM, Heabah NAE: Medulloblastoma: clinicopathological parameters, risk stratification, and survival analysis of immunohistochemically validated molecular subgroups. J Egypt NatlCancInst 33(1):6, 2021
19. Sarkar C, Deb P, Sharma MC: Medulloblastomas: new directions in risk stratification. Neurol India 54(1):16-23,2006
20. Giordana MT, Cavalla P, Dutto A, Borsotti L, Chiò A, Schiffer D: Is medulloblastoma the same tumor in children and adults? J Neurooncol. 35(2):169-761997
21. Pietsch T, HaberlerC : Update on the integrated histopathological and genetic classification of medulloblastoma – a practical diagnostic guideline. Clinical Neuropathology 35(6) : 344-35, 2016
22. Orr BA: Pathology, diagnostics, and classification ofmedulloblastomaBrain Pathology 30: 664–678, 2020
23. Sun Y, Sun XH, Fan WJ, Jiang XM, Li AW: Icariin induces S-phase arrest and apoptosis in medulloblastoma cells. Cell Mol Biol (Noisy-le-grand). 62(4):123-219. 2016
24. Ellison D: Classifying the medulloblastoma: insights from morphology and molecular genetics. NeuropatholApplNeurobiol. 28(4):257-282, 2002
25. Giangaspero F, Rigobello L, Badiali M, Loda M, Andreini L, Basso G, Zorzi F, Montaldi A.: Large-cell medulloblastomas. A distinct variant with highly aggressive behavior. Am J SurgPathol. 16(7):687-93, 1992

26. Maurer AJ, Bonney PA, Toho LC, Glenn CA, Agarwal S, Battiste JD, Fung KM, Sughrue ME. Tumor necrosis-initiated complement activation stimulates proliferation of medulloblastoma cells. Inflamm Res. 64(3-4):185-92, 2015
27. Maire JP, Guérin J, Rivel J, San Galli F, Bernard C, Dautheribes M, Caudry M. Le :médulloblastome de l'enfant. Incidence pronostique de l'hyperplasievasculaire de la nécrose de coagulation et de l'étatclinique post-opératoiresur la survie [Medulloblastoma in children. Prognostic incidence of vascular hyperplasia, coagulation necrosis and postoperative clinical state on survival]. Neurochirurgie. 38(2):80-88,1998.
28. Tural S, Gercek A, Konya D,Ozgen S, Toplamoglu H, Ozek MM:Microvessel density and vascular endothelial growth factor expression as predictors of childrens' survival from cerebellar medulloblastoma. J ClinNeurosci 16(9):1199-202,2009
29. Giangaspero F, Wellek S, Masuoka J, Gessi M, Kleihues P, Ohgaki H. Stratification of medulloblastoma on the basis of histopathological grading. ActaNeuropathol 112(1):5-12, 2006
30. Wippold FJ 2nd, Perry A: Neuropathology for the neuroradiologist: rosettes and pseudorosettes. AJNR Am J Neuroradiol27(3):488-92, 2006
31. Min HS, Lee YJ, Park K, Cho BK, Park SH: Medulloblastoma: histopathologic and molecular markers of anaplasia and biologic behavior. ActaNeuropathol 112(1):13-20, 2006.
32. Salaroli R, Ronchi A, Buttarelli FR, Cortesi F, Marchese V,Della Bella E, Renna C, Baldi C, Giangaspero F, CenacchiG:Wnt activation affects proliferation, invasivenessand radiosensitivity in medulloblastoma. J Neurooncol121:119–127, 2015
33. Goschzik T, ZurMühlen A, Kristiansen G, Haberler C, Stefanits H, Friedrich C, von Hoff K, Rutkowski S, Pfister SM, Pietsch T: Molecular stratification of medulloblastoma: comparison of histological and genetic methods to detect Wnt activated tumours. NeuropatholApplNeurobiol41(2):135-44, 2015
34. Goschzik T, ZurMu¨hlen A, Kristiansen G, Haberler C, StefanitsH, Friedrich C, von Hoff K, Rutkowski S, Pfister SM,Pietsch T: Molecular stratification of medulloblastoma:comparison of histological and genetic methods todetect Wnt activated tumours. NeuropatholApplNeurobiol41:135–144, 2015
35. Northcott PA, Jones DT, Kool M, Robinson GW, Gilbertson RJ,Cho YJ, Pomeroy SL, Korshunov A, Lichter P, Taylor MD,Pfister SM: Medulloblastomas: the end of the beginning. Nat Rev Cancer 12:818–834, 2012
36. Guessous F, Li Y, AbounaderR :Signaling pathways inmeduloblastoma. J Cell Physiol 217:577–583,2008
37. Utsuki S, Oka H, Sato Y, Tsutiya B, Kondo K, Tanizaki Y, Tanaka S, Fujii K. E, N-cadherins and beta-catenin expression in medulloblastoma and atypical teratoid/rhabdoid tumor. Neurol Med Chir (Tokyo) 44(8):402-406, 2004
38. Noronha, C.; Ribeiro, A.S.;Taipa, R.; Castro, D.S.; Reis, J.; Faria,C.; Paredes, J: Cadherin Expressionand EMT: A Focus on Gliomas.Biomedicines 9: 1328,2021
39. Huang, Z., Fan, G., Wang, D.:Downregulation of calbindin 1, a calcium-binding protein, reduces the proliferation of osteosarcoma cells. Oncology Letters 13.5: 3727-3733, 2017