Pediatric Medulloblastoma: Molecular biology, correlation with histopathological and clinical outcome

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By

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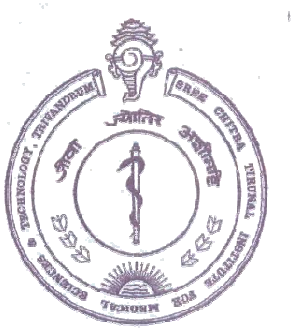

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CERTIFICATE

This is to certify that the thesis entitled "Pediatric Medulloblastoma: Molecular biology, correlation with histopathological and clinical outcome; is a bonafide work of Dr.**Amit Kumar Upadhyay** and was conducted in the Department of Neurosurgery, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram (SCTIMST), under my guidance and supervision.

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DECLARATION

This thesis titled "Pediatric Medulloblastoma: Molecular biology, correlation with histopathological and clinical outcome,is a consolidated report based on a bonafide study of the period from January 2006 to December 2007, done by me under the Department of Neurosurgery, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram.

This thesis is submitted to SCTIMST in partial fulfillment of rules and regulations of MCh Neurosurgery examination.

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Introduction

Most children diagnosed with cancer today are expected to be cured. Medulloblastoma, the most common pediatric malignant brain tumor, is an example of a disease that has benefitted from advances in diagnostic imaging, surgical techniques, and radiation therapy and combination chemotherapy over the past decades. An incurable disease 50 years ago, approximately 70% of children with medulloblastoma is now cured of their disease. However, the pace of increasing the cure rate has slowed over the past two decades, and we have likely reached the maximal benefit that can be achieved with cytotoxic therapy and clinical risk stratification. Long-term toxicity of therapy also remains significant. To increase cure rates and decrease long-term toxicity, there is great interest in incorporating biologic "targeted" therapy into treatment of medulloblastoma, but this will require a paradigm shift in how we classify and study disease.

The surge of advancement in genome analysis technologies has accelerated our understanding of molecular basis of MB. MB is no longer considered as a single disease. Using genome-based high-throughput analytic techniques, several groups have independently reported methods of molecular classification of medulloblastoma within the past year. This has resulted in a working consensus to view medulloblastoma as four molecular subtypes including WNT pathway subtype, SHH pathway subtype, and two less well-defined subtypes, Group 3 and Group 4. Each of them is characterized by discrete clinical presentation, demographic features, prognosis, expression profiling and genomic abnormalities. The identification of molecular subgroups has a great impact on clinical management, including patient stratification, treatment strategy, and design and implementation of targeted therapy..

Novel classification and risk stratification based on biologic subtypes of disease will form the basis of further study in medulloblastoma, and identify specific subtypes which warrant greater research focus. The identification of the differences in genetic alterations and prognosis among MB subgroups has added great significant clinical implications in future clinical management. It has been proposed that subgroups identification would be run in clinical setting so that patients with less aggressive tumors would be given deescalating intensive therapy to reduce cognitive and endocrine side effects. In contrast, patients with more aggressive tumors would be given an intensive therapy to enhance survival.

This study is such an attempt to classify a small subset of patients based on their molecular subtypes and correlate their outcome with the molecular subtype.

Review of literature

Medulloblastoma is the most common malignant pediatric posterior fossa neoplasm[1]; accounting for 20 to 25 % of all childhood brain tumors.[2-5]Medulloblastomas are undifferentiated embryonal neuroepithelial tumors of the cerebellum. Arising predominantly from the cerebellar vermis and primarily affecting children in the first decade of life. It can locally infiltrate the brain stem and / or the fourth ventricle and may spread along the craniospinal axis. MB typically arises in the midline cerebellum, in the region of the mid and inferior vermis[8,13].The tumor may grow to occupy much of the fourth ventricle, where it blocks circulation of cerebrospinal fluid (CSF) and causes hydrocephalus. MB may spread along the cerebellar peduncles and extend upward through the tentorial hiatus or downward into the cervical spinal canal. Brainstem invasion may also occur. In addition, up to 30% of patients have metastatic disease at diagnosis.[14]

Incidence

Medulloblastomas affect just under 2 people per million per year, and affect children 10 times more than adults. When looking at an estimated 68,530 primary brain and central nervous system tumors for 2012 in the USA, between 2005 and 2009 embryonal tumors (of which medulloblastomas are the majority) represented about 3,707 cases and of which 2,617 were in children between 0 and 19 years of age during the stated period. Medulloblastoma is the second most frequent brain tumor in children after Pilocytic astrocytoma and the most common malignant brain tumor in children, comprising 14.5% of newly diagnosed cases.

The incidence of childhood medulloblastoma is higher in males (62%) than

females (38%), a feature that is not seen in adults. The male- to-female ratio is approximately 2:1[2,12]. Medulloblastoma and other PNET`s are more prevalent in younger children than older children. 40% of medulloblastoma patients are diagnosed before the age of 5, 31% are between the ages of 5 and 9, 18.3% are between the ages of 10 and 14, and 12.7% are between the ages of 15 and 19. The median age at diagnosis is approximately 6 to 9 years.[6,7-9] Although MB is typically considered a pediatric malignancy, up to 30% of cases occur in adulthood.[10] In adults, the annual incidence has been estimated to be 0.5 case per million per year.[11]

Pathology

The cell of origin and the exact histological classification of this highly malignant tumour are still controversial. Although the majority occur as sporadic cases, hereditary conditions have been associated with medulloblastoma, including (1) Gorlin syndrome (nevoid basal cell carcinoma syndrome), (2) blue rubber-bleb nevus syndrome, (3) Turcot syndrome (e.g. glioma polyposis syndrome), and (4) Rubenstein- Taybi syndrome. The most frequent cytogenetic abnormality in sporadic medulloblastoma is an Isochromosome 17q [i (17q)]. Of tumors analyzed, 40-50% have a deletion of the short arm of chromosome 17, implicating the presence of a tumor suppressor gene that maps to 17p, which is distinct from the p53 gene. The nuclear protein p53 is thought to represent a tumor suppressor gene and has been mapped to the short arm of chromosome 17. Recently, Raffle et al. have shown that medulloblastomas have p53 mutations as identified by cDNA sequencing, suggesting inactivation or faulty transcription of the active protein encoded for by p53. These p53 mutations may be important in the pathogenesis of human medulloblastoma.

Baily and Cushing (1925) first described medulloblastoma as an invasive embryonal tumor of CNS arising from undifferentiated neuroepithelial cells in the cerebellum, initially labeled as "spongioblastomacerebelli."[15] Later, medulloblastoma and histologically similar tumors occurring outside the posterior fossa come under the umbrella term primitive neuroectodermal tumor(PNET) of the posterior fossa (Rorke 1983). [16]

Medulloblastoma is now classified as WHO grade IV tumor. In current WHO classification (Louis et al 2007), five distinct histopathological variant of medulloblastoma have been recognized.[8]

- Classic medulloblastoma
- Desmoplastic/ nodular medulloblastoma
- Medulloblastoma with extensive nodularity
- Anaplastic medulloblastoma and
- Large cell medulloblastoma

On gross pathologic inspection, MB appears as a pinkish gray to purple mass, commonly arising from the medullary velum and deriving most of its blood supply from the posterior inferior cerebellar artery. Some lesions are firm, discrete masses, whereas others are soft and friable. In a minority of cases, the tumor invades the floor of the fourth ventricle. Occasionally, gross hemorrhage is seen. CSF dissemination may result in a white, "sugar- coated" appearance of the cerebellar surface.

Classic medulloblastoma

About 80% of MBs are not classified as variants in the WHO scheme, being regarded as the classic form.

Microscopically, classic MB appears as a dense sheet of small, basophilic cells with little cytoplasm and round to ovalhyper chromatic nuclei (Fig. 1A).This has high nuclear: cytoplasmic ratio and a capacity to invade adjacent brain and leptomeninges. A high mitotic index may be seen. Evidence of differentiation along the neuronal or glial lineage is noted in up to 50% of cases.[17] Homer-Wright rosettes are commonly present.[8] There is a range of cell sizes in classic MBs. Occupying one end of this spectrum are tumours consisting of small cells with regular round or oval nuclei. The average area of these nuclei is 2–3 times less than the mean nuclear area in large-cell MBs at the opposite end of the range.

Desmoplastic/ nodular-

The desmoplastic/nodular MB subtype accounts for approximately 20% of cases, although it has been shown to account for as little as 5% and as many as 57% depending on the patient population examined.[18] Grossly, this variant is often located more laterally within the cerebellar hemisphere and may exhibit pial invasion. Microscopically, it is characterized by pale, reticulin- free nodules surrounded by reticulin-positive collagen fibers(Fig. 1B and C). These nodules represent regions of more advanced neuronal differentiation and they have reduced nuclear: cytoplasm ratio. They also have low mitotic activity and increased apoptosis.

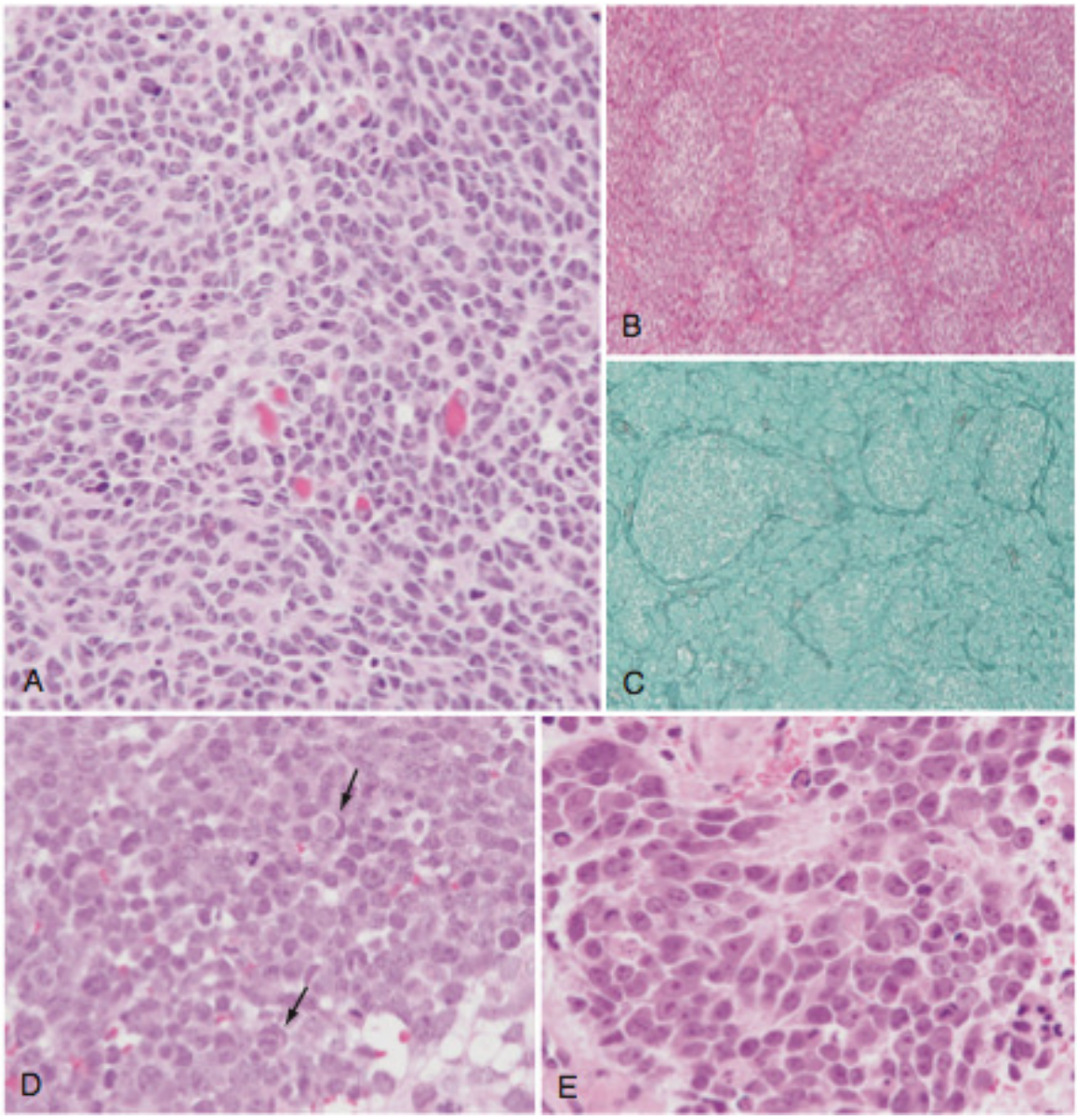


Figure-1- showingmicroscopic feature of various histological types of medulloblastoma

In the current edition of the WHO classification, two new MB variants were introduced: anaplastic MB and medulloblastoma with extensive nodularity (MBEN).[8,19]

Medulloblastoma with extensive nodularity(MBEN)

Large nodules, some appearing ovoid or reniform, dominate the histopathology of the MB with extensive nodularity (MBEN). This is a distinctive tumour that in the past has been labeled 'cerebellar neuroblastoma'. It represents the most differentiated form of the nodular/desmoplastic MB.When compared with the Desmoplastic/nodular variant, the reticulin-positive internodular zones are markedly reduced· Cells in its nodules show neurocytic differentiation, adopt a laminar pattern and are set against a neuropil-like background. A neuronal immunophenotype is characteristic.MBEN typically occurs in patients younger than 3 years and associated with favorable prognosis

Large cell/Anaplastic (LC/A)-

The large-cell MB has emerged as a variant with significant clinical and molecular associations, being associated with a poor prognosis and dissemination of tumour cells in CSF pathways at presentation. One difficulty in practice is that no clear-cut dividing line exists between some classic and large-cell MBs. The concept of anaplasia is relatively new. Anaplastic MBs are characterized by markedly atypical cells having angular pleomorphic nuclei with coarse chromatin wrapping around each other, with frequent moulding.[8,19] (Fig. 1D and E).

Earlier studies suggested that anaplasia was only confined to the large

cell variant of MB. However, recent studies have shown that they can also arise by malignant progression of classic and desmoplastic MBs. These cells are thought to represent focally aggressive clones capable of undergoing malignant progression, based on the observation that they often co-exist focally within MBs, or manifest only after recurrence or metastasis. Further patients with tumors having moderate to severe anaplasia (anaplastic group) had significantly shorter event-free survival and overall survival as compared to those with slight to no anaplasia (nonanaplastic group). The 5-year survival probability was 42% in patients with anaplastic variant in contrast to 68% for patients with nonanaplastic disease.

Clinical Presentation

- **Hydrocephalus**
    - Patients with medulloblastoma most commonly have symptoms related to increased intracranial pressure (as a consequence of hydrocephalus). Symptoms usually precede presentation by no more than 2 months.
    - Presenting symptoms are related to the age of the patient.
        - The younger, nonverbal patient presents with behavioral change.
        - Symptoms in younger children include listlessness, irritability, vomiting, and decreased social interactions.
        - Older children and adults complain of headache, especially upon awakening in the morning.
    - Vomiting without nausea is more common in the morning, since being recumbent (e.g. sleeping) increases intracranial pressure.
    - Often, symptoms of headache and vomiting prompt an extensive and lengthy workup of the gastrointestinal tract prior to consideration of the CNS.
    - Patients may develop double vision as the sixth cranial nerve becomes stretched from the hydrocephalus. Visual disturbances more commonly are a result of papilledema.
- **Cerebellar symptoms**
    - Most commonly found in children, the tumor involves the cerebellar vermis and causes gait ataxia more readily than unilateral symptoms.

- Adults more commonly harbor the desmoplastic variant of medulloblastoma, which arises in the cerebellar hemisphere. These patients often have symptoms of ipsilateral dysmetria.
- Head tilt and neck stiffness, caused by meningeal irritation, are complications of tonsillar herniation below the foramen magnum.
- Alternatively, head tilt can result from trochlear nerve palsy caused by direct tumor compression.

- **Leptomeningeal dissemination**
  - Presenting symptoms rarely are related to dissemination of tumor in the CSF.
  - Patients can complain of severe weakness from tumor compression of the spinal cord or nerve roots (e.g. radiculopathy).

- **Physical examination findings**
  - Increasing head circumference often is the only presenting symptom in infants.
  - These infants have full anterior fontanelles with widely split cranial sutures.
- **Ophthalmological examination**
  - Visual difficulty usually is due to papilledema; however, it also may originate from cranial nerve palsy.
  - Some studies have found papilledema (the most common physical finding) to be present in as many as 90% of patients.
  - As a consequence of hydrocephalus, the sixth cranial nerve can be compressed at the petroclival ligament, resulting in diplopia and lateral gaze paresis.

- Fourth cranial nerve palsy can be detected on careful extra ocular examination and should be considered in any patient with a head tilt.
- Patients with fourth cranial nerve dysfunction have greatest difficulty when eyes are rotated medially and depressed (eg, going down stairs). The fourth cranial nerve usually is compressed by direct tumor extension into the cerebral aqueduct.

- Examination of the extra ocular muscles may detect nystagmus, which, although nonspecific, can be related to a lesion of the cerebellar vermis.
- **Cerebellar signs**
  - Medulloblastoma most commonly is located midline. Therefore, unilateral dysmetria is less common than either truncal ataxia or a wide-based gait. Latter symptoms are easily observable on tandem gait.
  - As stated previously, desmoplastic medulloblastoma is more common in adults and usually arises in the cerebellar hemisphere.
  - Signs of ipsilateral cerebellar dysfunction in the arm or the leg are more common in this subtype.
- **Torticollis:** Head tilt can be a manifestation of either foramen magnum involvement or fourth cranial nerve palsy.

Radiology

The vast majority (94%) of medulloblastomas arise in the cerebellum and the majority of these, from the vermis (75%). They tend to protrude into the fourth ventricle from its roof, and may even grow directly into the brainstem. Other areas are uncommon, and are seen more frequent in older children and adults. In such cases the tumour is also more likely to be poorly marginated and demonstrate larger cyst formation. Adult medulloblastomas are usually located laterally, in the cerebellar hemispheres, with only 28% centered in the vermis.

Many of its imaging characteristics can be remembered by thinking of medulloblastoma as a small round blue cell tumour. It is also worth remembering that when these tumours are found in an atypical age group (i.e. older children or adults) then the appearance and location will be atypical also.

CTScan -

On CT, medulloblastomas appear as a mass arising from the vermis, resulting in effacement of the fourth ventricle / basal cisterns and obstructive hydrocephalus. They are usually Hyperdense (90%) and cysts formation / necrosis is common (40 - 50%), especially in older patients. Calcification is seen in 10 - 20% of cases. Enhancement is present in over 90% of cases and is usually prominent.

MRI-

- **T1** - hypo intense to grey matter
- **T1 - Contrast (Gadolinium)** - 90% enhance, often heterogeneously
- **T2 -** Heterogeneous due to calcification, necrosis and cyst formation

- Overall are iso to hyper intense to grey matter

- **FLAIR** - hyper intense to surrounding brain
- **DWI** - shows restricted diffusion
- **MR spectroscopy** - elevated choline, NAA decreased, may show a taurine peak

MRI is able to delineate the fourth ventricle and subarachnoid space to a much greater degree than CT. Although medulloblastomas project into the fourth ventricle, unlike ependymomas they do not usually extend into the basal cisterns.

As CSF seeding is common at presentation, imaging with contrast of the whole neuraxis is recommended to identify drop metastases and leptomeningeal spread. Although rare, extra neural spread is reported. Of medulloblastoma patients, 10-30% demonstrate CSF dissemination at diagnosis, mandating evaluation of the entire neuraxis with contrast-enhanced studies. Extra-axial metastases account for 5% of cases; most metastases are to the bone; less frequently, metastases are to the liver and lymph nodes.

Risk stratification

Currently medulloblastoma has been classified into standard risk and high-risk patient according to three criteria; age, extent of tumor resection and presence of metastasis at diagnosis. Currently, average-risk patients are defined as children 3 years and older without evidence of gross or microscopic metastatic disease at diagnosis and with less than 1.5 cm2 of residual tumor after surgical resection. High-risk patients include those younger than 3 years or any patient with evidence of metastatic tumor spread or significant residual tumor (>1.5 cm2) after surgery. Both histopathological and molecular variable are not included in this disease classification.

Despite its clinical utility, a criticism of the current staging system is its inability to differentiate between average-risk patients who may benefit from more aggressive intervention to maximize survival or less aggressive treatment to minimize the deleterious consequences of therapy. The identification of additional clinical, histologic, and molecular prognostic markers may improve the ability to estimate prognosis and direct risk-adapted therapy for MB patients.

Adding histopathological and molecular criteria to the current clinical classification may allow for better and more individualized disease staging leading to tailored therapeutic protocol, which may increase survival whilst minimizing the adverse effect of therapy.

Management strategies

Standard therapy for medulloblastoma consists of aggressive surgery followed by radiation to the entire craniospinal axis with boost to both the primary tumor site and focal CNS metastatic sites. Recently, adjuvant chemotherapy has also been shown to be beneficial.

Radiation Therapy

Average-risk disease

Reducing the amount of craniospinal radiation in an attempt to decrease morbidity without jeopardizing survival appears to be successful in this group. In a report by the International Society of Pediatric Oncology, children with average-risk medulloblastoma randomly received either the standard 36 Gy or a reduced dose of 24 Gy to the neuraxis.It was found that no statistical difference in progression-free survival rates was demonstrated between the groups as long as the initiation of radiotherapy was not delayed by the administration of chemotherapy before radiation.

The dose for average-risk medulloblastoma patients enrolled on Children's Oncology Group (COG) last completed trial was 23.4 Gy to the craniospinal axis followed by 32.4 Gy boost directly to the primary tumor site.In both the poor-risk and average-risk groups, the total radiation dose to sites of known disease is 55.8 Gy. An ongoing study is investigating further reduction of the craniospinal dose to 18 Gy in a subset of children with average-risk disease.

Poor-risk disease

The current recommendation is 36 Gy to the craniospinal axis, followed by a boost of 19.8 Gy to the primary tumor site and an additional 19.8 Gy to focal

metastatic sites. The amount of boost that can be given is limited by the presence of the optic nerves within the radiation field or if more than two thirds of the supratentorial compartment volume is within the radiation field.

Spinal disease that is visible after 30.6 Gy of the prescribed 36 Gy to the craniospinal axis receives an additional boost up to a total of 45 Gy if the tumor is located above the termination of the spinal cord and as much as 50.4 Gy if the tumor is located below the termination of the cord.

Infants

Radiotherapy for patients younger than 3 years, the poorest risk group, remains controversial. Because the effects of radiotherapy on intellectual development are most severe in this age group, attempts have been made to delay or omit radiation by using chemotherapy. However, in the most recent COG study, infants receiving chemotherapy alone had a 29% 3-year progression-free survival rate for those without dissemination and only 11% for those with metastasis. The Pediatric Oncology Group (POG) reported that, in infants with medulloblastoma treated initially with chemotherapy followed by delayed radiation, the 2-year progression-free survival rate was 34%.

Trials are currently underway to avoid or delay radiotherapy in this population by using cycles of high-dose chemotherapy followed by autologous stem cell rescue. Initial reports have indicated a good response rate to chemotherapy, and, although overall survival (30-40%) is comparable to prior studies, most patients who survived in the latest trials did not receive radiotherapy. Infants with desmoplastic tumor treated with chemotherapy fare better than those with classic tumors because 70% or more can be successfully treated without radiotherapy.

Chemotherapy-

Average-risk disease

The most encouraging results with adjuvant chemotherapy have been reported in children with nondisseminated medulloblastoma receiving 8 cycles of lomustine (CCNU), vincristine, and cisplatin chemotherapy for approximately 1 year following conventional dose radiotherapy and concomitant vincristine.

Latest trials indicate that children aged 3-10 years who received this regimen with reduced-dose craniospinal radiation have a superior survival rate compared to those who received standard radiation alone. The current 3-year progression-free survival rate for those receiving adjuvant chemotherapy is approximately 80%.

Poor-risk disease

Chemotherapeutic agents that have been found to be most effective for this disease are cisplatin, carboplatin, cyclophosphamide, and vincristine.

To improve survival rates in this group, current trials are investigating the use of high-dose chemotherapy (most commonly using carboplatinum and thiotepa-containing regimens) and autologous stem cell rescue after a course of conventional craniospinal radiotherapy and chemotherapy.

Studies using chemotherapy (carboplatin and vincristine) concurrent with radiotherapy are also underway.

Retinoic acid as a maturation agent following radiation is under investigation in a randomized trial.

Infants

In children younger than 3 years, evidence suggests that some do respond, at least partially, to chemotherapy. In patients with minimal residual postoperative disease, this response may be long lasting.

Ongoing trials are investigating high-dose chemotherapy (carboplatin and thiotepa) and stem cell rescue, following induction with chemotherapeutic agents similar to those used in the treatment for older children with poor-risk disease. Whether radiotherapy can be safely delayed or omitted altogether in certain subgroups has not yet been determined.

Methotrexate, both intrathecally and intravenously, is being added to more conventional chemotherapy in some studies; primarily for infants with partially resected and/or disseminated tumors.

Relapsed disease

Current studies investigating the use of biologic agents that specifically target the most common molecular alterations described in this disease, such as tyrosine kinase inhibitors that block the function of *EBB2,* are ongoing

Molecular subtyping of medulloblastoma

Recent studies have greatly improved the understanding of MB oncogenesis through elucidation of several developmental signaling pathways. These pathways involve cell signaling receptors, intracellular second messengers, transcription factors, and gene regulation. Tight regulation of these signaling cascades is essential to normal cerebellum development. Dysregulation of these pathways has linked to MB tumorigenesis.

The identification of molecular subgroups has a great impact on clinical management, including patient stratification, treatment strategy, and design and implementation of targeted therapy. Recent advancement in genome analysis technologies has led to categorize medulloblastoma in the 4 molecularly distinct subgroups, each having discrete clinical presentation, demographic features and disease outcome.

- WNT group
- SHH group
- Group 3
- Group 4

WNT group-

The best known of the medulloblastoma subgroups is the Wnt subgroup due to its very good long-term prognosis in comparison to other subgroups.[20-27]

Long-term survival rates for patients with Wnt medulloblastoma likely exceed 90%, with those patients who die succumbing more often to complications of therapy

or secondary neoplasms rather than to recurrent Wnt medulloblastoma [28].

The first evidence of demonstrating the involvement of WNT signaling pathway in MB came from genetic study of patients affected by Turcot syndrome, who have a 92- fold higher relative risk of developing MB than the general population. These patients carried a germ-line mutation of the adenomatous polyposis coli (*APC*) gene in the WNT pathway [29]. Subsequently, a small subset of sporadic MB was showed to harbor mutation of genes essential in WNT pathway. These included *APC,* B-catenin, (*CTNNB1*), and axin *1* (*AXIN1*) [30-34] Wnt medulloblastomas shows nuclear immunohistochemical staining for B-catenin.

Activation of WNT requires the interaction of Wingless (WNT) ligand. In the absence of ligand Wingless, the key downstream effector, CTNNB1, is undergone ubiquitination and degradation. This pathway is activated when the ligand binds to a receptor complex composed of a seven trans membrane Frizzled (FZ), serpentine receptor and low-density lipoprotein receptor-related protein (LRP). This leads to phosphorylation of disheveled (DVL), association with AXIN, and prevention of CTNNB1 phosphorylation by glycogen synthase kinase- 3B(GSK-3B)[2]. The stabilized CTNNB1 is then translocated to the nucleus where it interacts with transcription factors T-cell factor (TCF) and lymphoid enhancer factor (LEF) to activate transcription of targets genes such as MYC, JUN, FRA, AXIN2, and CCND1[30-42]

Nearly all of the Wnt medulloblastomas studied to date have classic histology. 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 [28]

Overall medulloblastoma is more common in males, however, the gender ratio for Wnt medulloblastomas is about 1:1 male: female. Wnt medulloblastomas can occur at all ages, but are uncommon in infants.

As most patients with Wnt medulloblastoma survive, 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.

Sonic hedgehog subgroup

SHH subgroup comprises approximately 25-30% of MB [43-45]. Individuals with germ line mutations in the Shh receptor PTCH have Gorlin syndrome, which includes a predisposition to medulloblastoma[46,47] Similarly, individuals with germ- line mutations of the Shh inhibitor SUFU are predisposed to medulloblastoma, particularly infantile medulloblastoma [48-51] Similarly, somatic mutations of PTCH, SMO, and SUFU, as well as amplifications of GLI1 and GLI2 have been found in sporadic medulloblastoma [52-54].

Sonic hedgehog subgroup medulloblastomas have largely been identified on the basis of transcriptional profiling[24,25,27,55-57]. Other approach to identify Shh medulloblastomas have included immunohistochemical staining for SFRP1 [25,27,58] or GAB1 [59]. At the chromosomal level, loss of 9q is the most frequent abnormality in SHH, accounting for 21-47% [60-63]. Other chromosomal aberrations found in this group of tumors include gain of chromosome 3q and 9p and loss of chromosome 10q, 20p and 21p [60,64]

SHH subgroup is characterized by the high frequency of desmoplastic histology (40%) although other variants are found in this subgroup [60,61,65,66]. The age

distribution of SHH subgroups display a bimodal shape, with majority of SHH in both infants (0-3 years) and adults (>16 years), but much less frequent in children (3-16 years) [65]. SHH subgroup comprises of half of the adult MB [67].

Some recent series [25], have found a preponderance of females among SHH medulloblastoma. Taken into account all published studies, the gender ratio is approximately 1:1.The prognosis of SHH tumors is good in infant and intermediate in adult [68].

Group 3 and Group 4

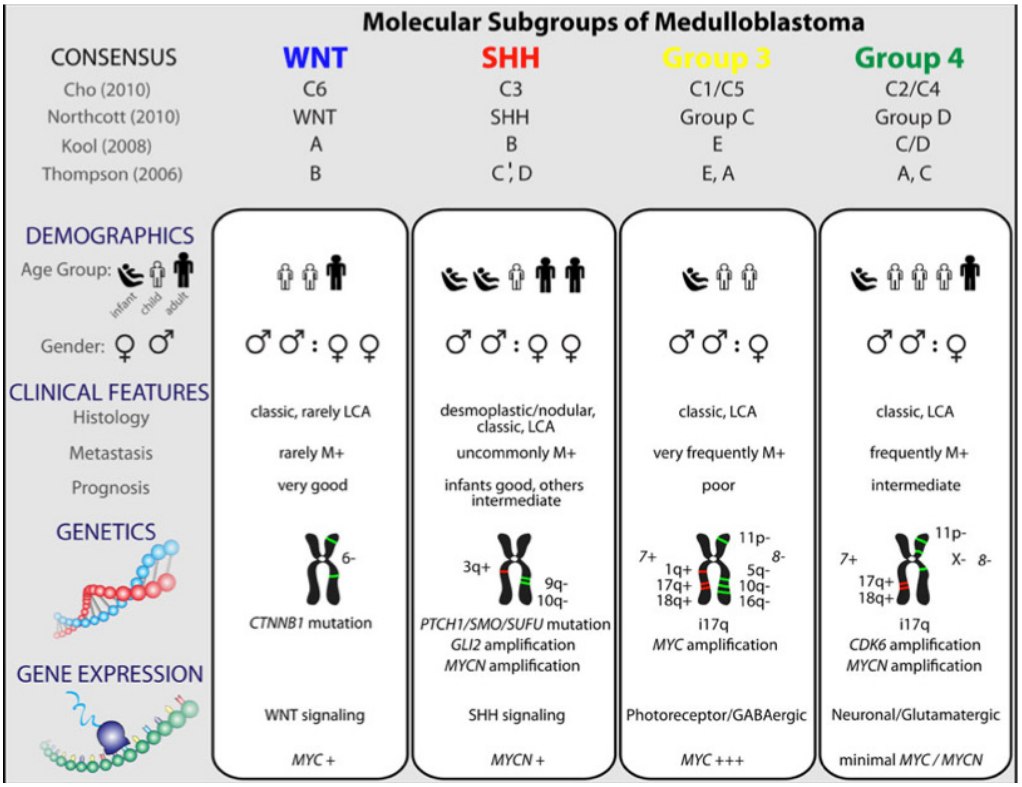
Groups 3 and 4 were originally so called the non-WNT/non-SHH groups. They share some of the similarities in both clinical presentation and molecular profiling. Most tumors of this group display classical histology. LC/A and desmoplastic histologies are present but at a lower frequency [61, 63,65, 66]. The age of onset is distributed in both groups with most cases are children [61,69]. Although both groups have similar frequency of metastasis, Group 3 exhibits poor prognosis, and Group 4 shows intermediate prognosis[63,68,70].

These two subgroups are more similar to each other, and some characteristics of Group 3 are also observed in Group 4 [63]. For instance, both subgroups are enriched for expression of genes involved in photoreceptor differentiation [62, 70]. Furthermore, they express high level of OTX2 and FOXG1B, well-known oncogenes of MB [63]. Nevertheless, Group 3 is distinguished by its enriched gene signatures functioned in cell cycle, protein biosynthesis, glutamate receptor signaling, and p38 mitogen-activated protein kinase (MMAPK) pathway. In contrast, Group 4 is overrepresented by genes involved in neuronal differentiation, neuronal development, cytoskeleton organization and biogenesis or vesicle mediated transport[62,63].

Isochromosome 17q (I17q) represents the most common structural abnormality in Groups 3 and Group 4, with higher incidence observed in Group 4 than in Group 3 (66% Vs. 26%)[63,65,70]. Other chromosomal aberration events identified in these subgroups include gain of 7 and 18q and loss of 8 and 11p [61,62,63,69.]

A main difference between Groups 3 and 4 is the enrichment of MYC amplification in group 3, a feature very rarely observed in Group 4, as well as WNT and SHH[63,65, 66]. Another difference is the enrichment of chromosome X loss in Group 4, which is observed at a frequency of 80% in female with Group 4 [62, 71].

Signaling pathways or biological programs driving the pathogenesis of Groups 3 and 4 still remain largely unknown. Two latest studies illustrate that genes regulated H3K27me3 (histone H3 lysine K27 trimethylation) are recurrently dysregulated in Groups 3 and 4, but not in SHH and WNT [65, 66].



Comparison of the various subgroups of medulloblastoma including their affiliations with previously published papers on medulloblastoma molecular subgrouping

Development of subgroup affiliation assays

Major challenge is the development of accurate subgroup affiliation assay for the establishment of molecular subgroups of MB. An optimal assay needs to be rapid and robust. In addition, it can be applied on common diagnostic materials, such as paraffin –embedded tissues. At present, two research groups have established immunohistochemical method for identification of molecular subgroups.

The first proposed affiliation assay involves immunohistochemical staining of a panel of antibodies. These include CTNNB1 or DKK1 for WNT, SFRP1 for SHH, NPR3 for Group 3, and KCNA1 for Group 4 [63, 72, 74].

Ellison et al. have also described another set of immunohistochemistry markers, namely, GAB1, CTNNB1, filamin A, and YAP1 for identification of WNT, SHH, and non-WNT/non-SHH [73].

WNT tumors show strong nuclear and cytoplasmic CTNNB1 staining. These tumors are immunoreactive to filamin A and YAP1, but not GAB1. SHH tumours display cytoplasmic staining of CTNNB1, and exhibit positive immunostaining for filaminA, GAB1, and YAP1. Non WNT/non SHH tumours are only present with cytoplasmic CTNNB1 staining and they are immunonegative for filaminA, GAB1, and YAP1.

Future directions and urgent clinical needs

As we begin the transition from clinical stratification to molecular stratification, it will be important in our opinion to re-join groups of patients that have historically been stratified based on clinical parameters. Even if nearly all children over age three are pooled as study subjects, randomized phase III trials are expected to take five to ten years to accrue sufficient numbers of patients. This is particularly true for those patient groups where modest improvements in outcome are possible or for efficacy equivalency studies, both of which require more patients to achieve statistically relevant endpoints.

Molecular classification of all medulloblastoma using a gene expression based approach will improve current clinical risk stratification, but require significant coordination and centralization of testing Use of novel technology to measure gene expression using paraffin embedded tissue may significantly improve feasibility in national consortium clinical trials. This approach will likely be coupled, at least initially, with reliable traditional pathological techniques including beta-catenin nuclear staining and fluorescent in situ hybridization to detect MYC amplification At a minimum, the practical application of this strategy in the next phase of clinical trial development would be to exclude patients with MYC amplified tumors from inclusion on trial arms studying therapy de-escalation such as the current standard risk trial.

There is a most urgent clinical need for further research into the biology of non-WNT, non-SHH medulloblastoma. Because driver mutations are not well understood, there are no published transgenic mouse models of this disease. An alternative strategy for mouse model development is the patient-derived xenograft in

which human tumor samples obtained at the time of therapeutic surgery are transplanted into animal models. This method, developed by the Li laboratory, provides resources for study and therapeutic evaluation for rare and incompletely understood subtypes of disease for which there are no transgenic models.

Aim of the project

The overall aim of this study was to identify and validate pathological and molecular markers in a cohort of 23 pediatric patients (age 0-18 years) with medulloblastoma and to analyze their role in outcome prognosis.

Subsidiary aims are:

- To develop methodologies for the routine assessment of molecular markers in routinely collected FFPE tumor material
- To determine relationship between molecular, pathological and clinical features assessed in this cohort, if any.
- To combine these data to develop optimal models for disease risk stratification in medulloblastoma, based on molecular, clinical and pathological variable.

Materials and Methods

Study cohort

Study involved retrospective analysis of a cohort of 23 consecutive patients in age group 0 to 18 years of age with histological diagnosis of medulloblastoma, who have undergone surgery between, January 2006 to December 2007 at our institute. Study material consists of histopathological paraffin blocks and slides archived in department of pathology at our institute.

Patients of age above 18 years and those, whose archival material was not suitable for molecular biology studies, were excluded from the study.

Histology and immunohistochemistry

Standard histological preparations (hematoxylin and eosin) were used to assess general architectural and cytological features, including nodule formation, differentiation along neuronal (neurocytic/ganglionic) and astrocytic lines, and large cell or anaplastic phenotypes.

Reticulin preparations were used to evaluate desmoplasia. Internodular desmoplasia was required for a diagnosis of desmoplastic/nodular (D/N) medulloblastoma, including the paucinodular D/N variant, and medulloblastoma with extensive nodularity (MBEN). The MBEN is defined by its large irregularly shaped nodules, pronounced internodular neurocytic differentiation, and sparse internodular desmoplastic regions

As defined by the WHO classification of CNS tumors and restated in criteria adopted for COG trials in North America, the anaplastic medulloblastoma shows

marked cytological pleomorphism across most of its area, in association with high mitotic and apoptotic counts. The large cell medulloblastoma is defined by its groups of uniform large round cells with a single nucleolus, in most cases admixed with groups of anaplastic cells. Large cell and anaplastic tumors were combined in study datasets as LC/A tumors

Molecular subgroups of disease into SHH, WNT, and non-SHH/WNT were done using standard antibodies specific for these pathways. We used anti-Sonic hedgehog antibody (EP1190Y), Ab53281 (Abcam) for SHH and anti-Wnt antibody ab85060 (Abcam) for Wnt pathway. Those that stained negative for both, we grouped them as Non Shh/Wnt group.

Tissues were fixed in 10% formalin and embedded in paraffin wax. Sections taken with a microtome were mounted on to poly-L-lysine coated slides. These were deparaffinised in two changes of xylene (10 minutes each). Subsequently the tissues were hydrated with 100% alcohol twice then followed by one wash each with 95% and 70% alcohol (5 minutes). After washing in tap water and deionized water (5 minutes each) the sections were treated for heat-induced epitope retrieval (HIER) using citrate buffer at pH 6.0 at 95°C for 10 minutes and then incubated with H_2O_2 for 30 minutes to quench endogenous peroxidase activity. Again the sections were washed with distilled water followed by blocking with serum for 10 minutes. The slides were then incubated at 4°C overnight with primary antibodies anti-Wnt-1 antibody (Abcam, 1:250), and anti-SHH antibody (Abcam, 1:100) diluted with 1% BSA in TBS. They were then washed in TBS and incubated with Goat anti-rabbit HRP Conjugate antibody for 30 minutes. Excess secondary antibodies were washed with TBS and stained with 3, 3' diaminobenzidine tetrahydrochlorideand

counterstained with Harris hematoxylin for one minute. Blueing is done initially with tap water for 10 minutes followed by an acid alcohol dip and finally with in ammonia water for 15 minutes. Sections were then dehydrated by treating with 70% and 95% alcohol (5 minutes each), two times with 100% alcohol (5 minutes each) and xylene (10 minutes each) and mounted with DPX.

Clinical outcome analysis-

Clinical outcome of the patients was analysed by retrospective analysis of five years follow up data from medical records. Parameters analysed were

- Age
- Sex
- Extent of resection of tumour
- Extent of residual disease
- Presence of CSF seeding
- Adjuvant treatment postoperatively

Clinical outcome was measured on Modified Rankin Score

Statistical Analysis

Associations among clinical features (sex, age at diagnosis, and clinical risk group) and histopathological and molecular characteristics were investigated using the chi-square test for multivariate analysis with discrete parameters. Overall survival (OS) was defined as the interval between start of therapy and date of death on study or last follow up in surviving patients.

Results

Patient's characteristic: -

There were a total of 23 pediatric cases (age 0-18 years);of these five were excluded, as the archived paraffin blocks were not suitable for immunohistochemical staining. Out of remaining 18 patients 7 were male and 11 were females (M: F ratio 1:1.57). Mean age at presentation was 6.22 years.

Recurrent vomiting was most common presentation in infants and younger children. In older patients headache associated with vomiting and gait ataxia were the predominant symptoms.

All patients underwent surgical decompression of the tumour followed by standard adjuvant treatment. Total or near total resection of the tumour was achieved in all cases and none of the patients had significant residual tumour in postoperative scan.

Two patients (11.11 %) had supratentorial metastasis within two years of resection of primary tumour and they required decompression of the metastasis.

None of the patient had major surgical morbidity and most were followed for more than 1 year after operation. The mean survival ranged from 1 month to 101 months (mean 30.16 months).

11/18 (61.11%) children had CSF seeding in craniospinal axis in preoperative gadolinium enhanced MRI at presentation. Mean survival in patients with CSF dissemination was 24.18 months (1to 101 months) and those without dissemination was 39.57 months (2 to 98 months).

Five out of 18 (27.78 %) patients were below 3 years of age. Mean survival in these patients was 13.8 months (range 1 month to 28 months). Mean survival in patients above 3 years of age was 36.46 % (1 month to 101 months).

Histopathological features and prognosis-

The review of the H&E stained section focused on nuclear size and anaplasia. We counted > 10 areas from each of the stained tissues and then averaged the counted values. Classical histology was most common with almost 50 % of the children having a classical medulloblastoma (Fig.2). It was characterized by relatively uniform sheets of undifferentiated, small round blue cell.Large cell (LC) and anaplastic (A) histology was grouped together in this study because all large cell medulloblastomas were associated with variable degree of anaplasia. Large cell/anaplastic (LC/A) tumour was present in 7 cases (38.89 %). It consisted of large, pleomorphic cells with prominent nucleoli, nuclear wrapping with variable mitotic and indices. The desmoplastic variant was present in onlyone and was characterized by sheets of small nodules surrounded by densely packed, highly proliferative cells and dense reticulin fiber network. Medulloblastoma with extensive nodularity (MBEN) was present in only one case and was characterized by grapelike large nodules surrounded by thin reticulin rich background.

Mean age at presentation was similar in classical and LC/A subgroups (6.6 years and 6.4 years respectively).Mean survival was 39.44 months (range-1 month to 101 months) in classical histology and 21.28 months (1 month to 74 months) in large cell/ anaplastic type. Patients with Desmoplastic and MBEN histology comprised one case each and they survived for 11 months and 28 months respectively (Table-1).

Table-1- showing mean age at presentation and mean survival of different histopathology group.

Histopathology		Age	Meansurvival
Classical	Mean	6.667	39.444
	N	9	9
	Std. Deviation	3.9686	42.8722
	Minimum	1.0	1.0
	Maximum	12.0	101.0
Desmoplastic	Mean	6.000	11.000
	N	1	1
	Std. Deviation		
	Minimum	6.0	11.0
	Maximum	6.0	11.0
LC/A	Mean	6.429	21.286
	N	7	7
	Std. Deviation	3.9521	27.8012
	Minimum	1.0	1.0
	Maximum	12.0	74.0
MBEN	Mean	1.000	28.000
	N	1	1
	Std. Deviation		
	Minimum	1.0	28.0
	Maximum	1.0	28.0
Total	Mean	6.222	30.167
	N	18	18
	Std. Deviation	3.8280	35.1806
	Minimum	1.0	1.0
	Maximum	12.0	101.0

Table 2- (ANOVA)comparing mean survival and age distribution in various histological subtypes

		Sum of Squares	df	Mean Square	F	Sig. (p value)
Age * Histopathology	Between (Combined) Groups	29.397	3	9.799	.624	.611
	Within Groups	219.714	14	15.694		
	Total	249.111	17			
Meansurvival * Histopathology	Between (Combined) Groups	1698.849	3	566.283	.410	.748
	Within Groups	19341.651	14	1381.546		
	Total	21040.500	17			

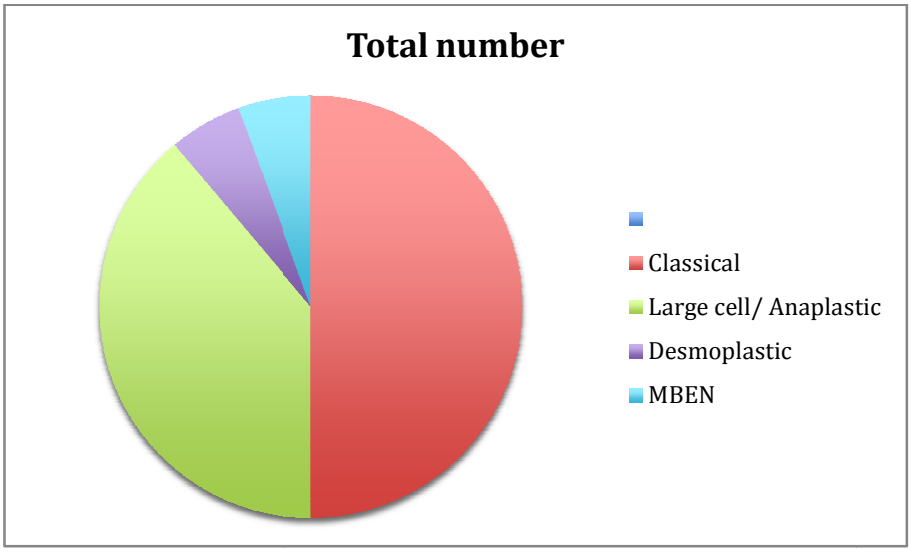


Fig. 2- Showing frequency of various histological types in cohort

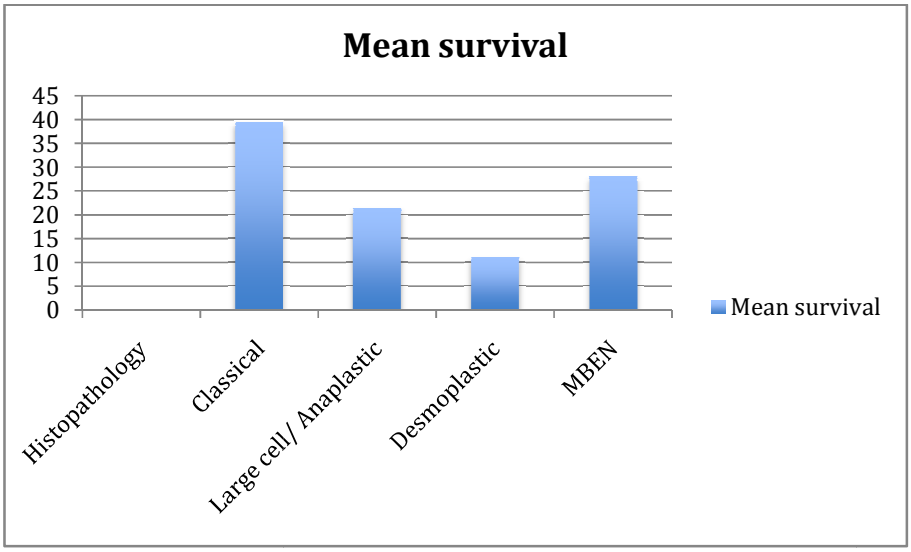


Fig.3-Showing mean survival of different histological subtypes

Molecular biology and prognosis: -

By using standard immunohistochemistry we found Wnt subgroup as most common subtype present in 55.56 % of the cases (Fig.4). Shh subgroup was present in 27.78%. Tissues, which were not stained positively for Wnt and Shh, were grouped as Non Shh/ Wnt subgroup and this consisted of 16.67% of the cases. Mean age at presentation was higher in Wnt group (7.4 years). Shh and Non Shh/Wnt subgroups had almost similar age at presentation (4.4 years and 5.3 years respectively) (Table 3).Non Shh/Wnt group was more common in males while other two groups were common in females. Mean survival was maximum in Wnt subgroup (38.3 months) (Fig.4) and Non Shh/Wnt showed worst survival with a mean of 4 months. Patients with Shh group had intermediate survival between above two groups (mean 29.6 months)

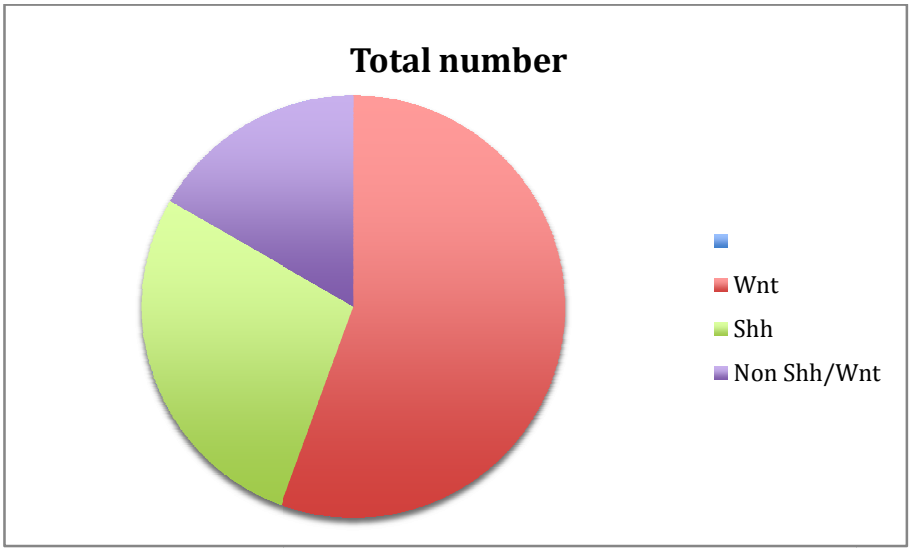


Fig.4- Showing frequency of various molecular types in our cohort.

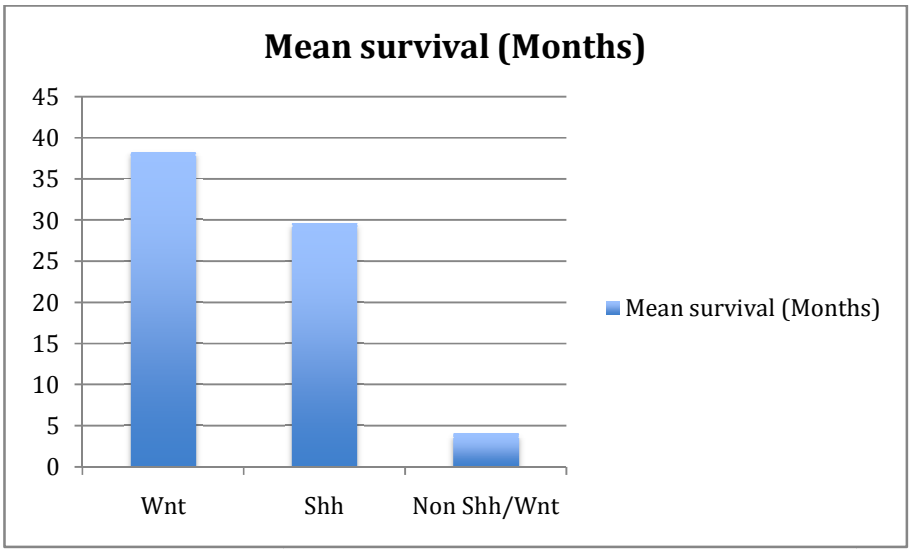


Fig.4- Showing survival of different molecular subgroups

Table 3 –Showing meant age at presentation and mean survival of different molecular subgroup.

Molecularsubtype		Age	Meansurvival
Non Shh/WNT	Mean	5.333	4.000
	N	3	3
	Std. Deviation	4.0415	3.4641
	Minimum	1.0	2.0
	Maximum	9.0	8.0
SHH	Mean	4.400	29.600
	N	5	5
	Std. Deviation	4.7223	38.9525
	Minimum	1.0	1.0
	Maximum	12.0	98.0
WNT	Mean	7.400	38.300
	N	10	10
	Std. Deviation	3.2387	36.8633
	Minimum	1.0	1.0
	Maximum	12.0	101.0
Total	Mean	6.222	30.167
	N	18	18
	Std. Deviation	3.8280	35.1806
	Minimum	1.0	1.0
	Maximum	12.0	101.0

Table 4- (ANOVA)-comparing mean survival and age distribution between various molecular subtypes.

			Sum of Squares	df	Mean Square	F	(p value) Sig.
Age * Molecular subtype	Between Groups	(Combined)	32.844	2	16.422	1.139	.346
	Within Groups		216.267	15	14.418		
	Total		249.111	17			
Meansurvival * Molecular subtype	Between Groups	(Combined)	2717.200	2	1358.600	1.112	.354
	Within Groups		18323.300	15	1221.553		
	Total		21040.500	17			

After analyzing the correlation of histological subtype and molecular subtype we found that the most common histological subtype in Wnt group was classical (60%) and second most common was LC/A type (30%) (Fig.5). MBEN histology was present in only one patient and corresponds to Wnt group. In Shh group, both LC/A and classical were present with equal frequency (40 % each) and one patient had Desmoplastic histology. In Non Shh/Wnt group total 3 cases were there out of which 2 (66.67 %) were LC/A type and one was classical histology.(Table 5)

	Wnt	Shh	Non Shh/Wnt
MBEN	1	0	0
Classical	6	2	1
Large cell/Anaplastic	3	2	2
Desmoplastic	0	1	0

Table-5- Showing frequency of different histological subtype in a particular molecular group

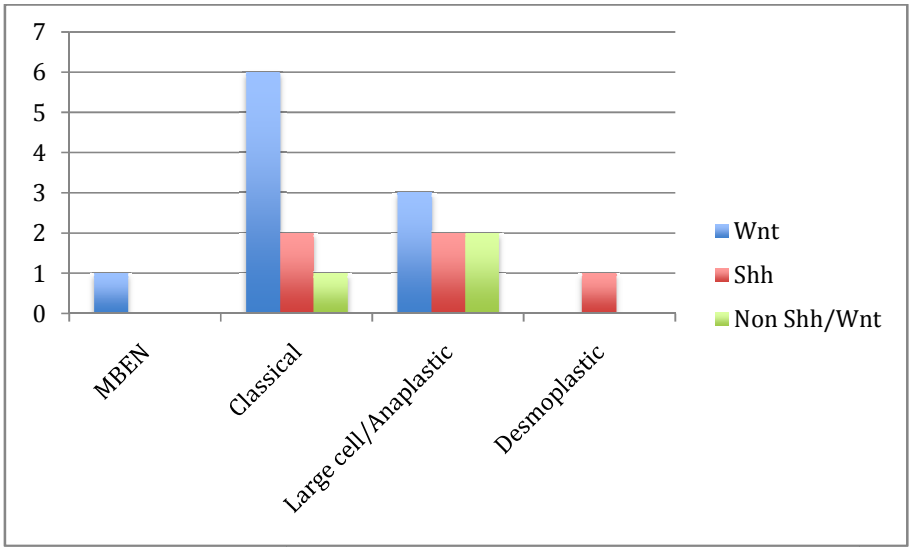


Fig. 5-Showing frequency of different histological subtype in a particular molecular group

Discussion

Medulloblastoma is the most common malignant brain tumour in children. It is a highly malignant neoplasm with a strong tendency for dissemination along the neuraxis and for the formation of subarachnoid and ependymal distant metastases. Since the mid-1990s, the risk classification for relapse and selection of treatment of MB patients has remained strictly clinical, with cases stratified into two-risk groups, viz. 'average risk' and 'high risk,' based on the following criteria: (i) age, (ii) extent of resection, and (iii) Chang metastasis staging i.e. Presence or absence of metastasis. This clinical staging has been helpful as a broad guide for predicting prognosis. However a major drawback is that this does not differentiate high- from low-risk patients within the same clinical stage. Although some reports in the literature have shown up to a 70% 5-year survival rate for some of these patients, their survival comes with the risk of significant long- term treatment-related morbidity. In our study all patients underwent maximal resection, craniospinal irradiation and chemotherapy. We found mean survival of 30.16 months, but there was significant difference in clinical outcome in patients with similar tumour staging, which allow us to learn about the biological heterogeneity of these tumours.

In our study we have observed no significant difference in overall survival of the patient with CSF seeding (M1) and that without CSF dissemination (M0) (24.18 months Vs. 39.57 months) in patients with similar clinical staging, which corroborate that biological behavior of the tumour also play a major role in predicting the survival of the patients with medulloblastoma.

WHO 2007 has classified medulloblastoma into 5 histological subgroups.

MBEN is the subtype, which is having best outcome among all histological subtypes. This is intriguing because it generally affects young infants who according to the clinical stage belong to the high-risk category. In our study the median survival of patients with age less than 3 years was 13.8 months, which was significantly less than mean survival of older children (36.46 months), validating the fact that age is an important prognosticating factor and should be included in risk stratification of diseases. Among patients less than 3 years of age, only one patient had MBEN histology and overall survival of that patient was relatively better than mean survival of other patients in that group. Although, because of lesser number, this is not statistically significant, this result favors the previous studies, which states that prognosis of MBEN histology is better[75,76]. Thus patients younger than 3 years, which has been stratified as high-risk group, can further be stratified into favorable and unfavorable outcome based on histology.

Difference in prognosis between the classic and desmoplastic variants remains controversial. Desmoplastic MBs have been variably correlated with better outcome[77-79] by some, while others found it to be either associated with worse prognosis,[80] or without any correlation with survival time.[81-83]

In our study only one patient had desmoplastic histology with overall survival 11months. This result is inadequate to comment prognosis of this histological subtype. Classical histology was most common histological type in this study, present in 50% of cases (Fig. 2).The LC/A group was next in frequency accounting about 38.89 %. In previous literature it has been mentioned that classical histology is most common accounting about 65 % and LC/A group is least common (about 10 %). Incidence of Desmoplastic falls in between (about 15%). Our results regarding

classical histology was similar to previous studies but LC/ A group was second most common type in present study. Desmoplastic histology is more common in older age group, this probably explains the paucity of this histology in present study. We found that classical histology had significantly better overall survival than LC/A histology (mean 39.44 and 21.28 months respectively), but this was not statistically significant ($p > 0.05$)(Table-2).Variation in survival in a particular histological group can be explained by degree of anaplasia present on histological examination. Earlier studies suggested than anaplasia was only confined to the large cell variant of medulloblastoma. However recent studies have shown that they can also arise by malignant progression of classic and desmoplastic medulloblastoma. [84-86]These cells are thought to represent focally aggressive clones capable of undergoing malignant progression, based on the observation that they often co-exist focally within MBs, or manifest only after recurrence or metastasis.[84,86-88]

Brown et al[89] in a review of 474 medulloblastomas from POG patients reported that the long-term survival of large-cell medulloblastoma with anaplasia was 10% compared to more than 50% in large-cell medulloblastoma without anaplasia.

In fact, on log-rank analysis, grade of anaplasia allowed better stratification of patients with respect to outcome than the current clinical stage, indicating that histological grading is not a surrogate for clinical staging, but rather an independent predictor of survival.

Now a days it is widely accepted that identification of molecular alteration allows a clinically relevant subgrouping of medulloblastoma with particular profile of biological behavior and outcome.

In our study we found Wnt as the most common molecular group on immunohistochemical staining, comprising (55.56%) of cases and Non Shh/Wnt as least common group (16.67%). Shh pathway was involved in 27.78 % cases (Fig 4).

We found that Wnt pathway was involved in relatively older patients (mean 7.4 years) however there was no significant difference in age at presentation among Shh and Non Shh/Wnt groups (4.4 years Vs. 5.33 years). On correlating the histology with molecular subtype, we found that classical histology was most common in Wnt pathway (60%) followed by LC/A histology, which comprised 30%. One patient had MBEN histology. In Non Shh/Wnt group, although there was lesser number of cases, we found LC/A histology as most common type (66.67%). One patient in this group had classical histological subtype. Historically desmoplastic histology has been considered a surrogate for activation of the Shh pathway, however, genomic data have indicated that this association is not reliable and other histologies (classical, LC/A and MBEN) have also been observed within this subtype. In present study, we found desmoplastic as least common histology in Shh group (20%), Classical and LC/A histology was present in 40% each. This finding was contradictory to previous study in which they mentioned Desmoplastic as most common histology in this group. This may be due to the fact that desmoplastic is more common in older age group and in our study we have selected only pediatric patients (age 0-18 years).

The mean overall survival in was best in Wnt subgroup. The worst survival was found in Non Shh/Wnt group, the worse prognosis in this group could be explained by large numbers of LC/A histology. We found that patients with LC/A histology, which belong to Wnt group, had relatively better overall survival than those patients with similar histology belong to Non Shh/Wnt group. This explains that

signaling pathway has significant impact on prognosis of the patients and this should be included in risk stratification of medulloblastoma.

Although till date no association has been found of alteration in Shh pathway with prognosis in medulloblastomas, an increasing number of anti-cancer drugs are being designed to target this pathway. Cyclopamine is one example of a plant-derived teratogen that specifically inhibits the Shh pathway in medulloblastoma cells causing anti-tumour activity. [90]

The assay of a molecular marker must be reliable, and its analysis undertaken on statistically meaningful cohort of tumour from patients treated in uniform manner. A valid test of any hypothesis that involves examining the prognostic value of a novel marker alongside clinical and pathological variables will require a multivariate survival analysis. These are stringent requirements for uncommon tumours like the medulloblastomas, but can be met in the context of multicenter clinical trials. Analyzing tumours from patients in such trials has several advantages; it is possible to recruit sufficient samples, the patients will have been treated according to a defined protocol, and the coordinating center can supply clinical data. In our study the overall results showed that both, molecular and histological subtype have significant impact on prognosis of medulloblastomas. However because of the small cohort, our results regarding survival in various histological and molecular groups was not statistically significant. We could not made any definite comment on incidence or survival of Desmoplastic or MBEN type because of lesser number of patients and paucity of these histological subtypes in this study. We need to include more number of cases to validate the results. Combining medulloblastomas from adults and children to increase the number is also problematic, because they tend to have been exposed to

different treatment protocols.

Even so, studies of the prognostic value of molecular markers in medulloblastomas too often rely on what is available in the archive of the local pathology department, which further limit the selection of cases. In our study we have to exclude five cases because the archived material was not suitable for histopathological examination. This can be overcome if we start doing immunohistochemical staining routinely on fresh frozen samples or implementing immunohistochemical staining as an integral part of routine histopathological examination in all cases of medulloblastoma. Implementing these strategy would provide more number of cases and we would be able to predict the prognosis and plan the further management accordingly, after surgery.

Limitations of our study

This study is essentially a pilot study to assess the feasibility of molecular characterization of medulloblastomas. The small number of patients precludes any major conclusions. Being the first attempt, our techniques of molecular characterization also were not standardized. Hence we encountered quite a number of overlaps with patient samples showing positivity for both Wnt and Shh pathways. We had to exclude those five samples from our study for the said reason.

Conclusion

Radical surgical excision and optimal adjuvant therapy has significantly revolutionized long-term outcome for children with medulloblastoma.

In addition to clinical and histopathological parameters, molecular pathway markers provide valuable prognostic clues.

Children with Wnt pathway have the best outcome and those belonging to the nonWnt/nonShh group have a poor outcome and Children belonging to the Shh group fall in between.

Our study is probably one of the earlier attempts at molecular subtyping in an Indian setting. Our study proves that molecular subtyping is a) feasible b) cost effective and c) a valuable add on in prognosticating outcome for children with medulloblastoma.

Further studies with larger number of patients and a longer follow up will provide more data to formulate guidelines for risk stratification based on molecular subtyping

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