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ORIGINAL ARTICLE

Molecular Subtyping of Paediatric Medulloblastoma by Immunohistochemistry

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ABSTRAK

Empat subkumpulan molekul teras medulloblastoma yang mempunyai profil transkripsi dan nilai prognostik yang berbeza diperkenalkan baru-baru ini. Kajian ini bertujuan untuk menentukan varian histologi dan subkumpulan molekul medulloblastoma melalui aplikasi imunohistokimia (YAP-1 dan beta catenin) sebagai penanda surrogate di populasi kami, di samping menghubungkan varian histologi dan subkumpulan molekul ini dengan parameter klinikopatologi. Kami telah melibatkan seramai tujuh belas pesakit medulloblastoma yang berusia empat bulan hingga 14.3 tahun dari tahun 2002 hingga 2017. Histologi klasik (76.5%) adalah histologi medulloblastoma yang paling umum, diikuti oleh varian sel besar/ anaplastik (LCA) (17.6%) dan desmoplastik/nodular (DN) (59%). Subkumpulan molekul yang paling kerap adalah tumor bukan SHH/WNT (64.7%), diikuti oleh tumor SHH (35.3%). Di antara tumor SHH, 66.7% adalah histologi klasik dan selebihnya 33.3% adalah varian LCA. Yang menariknya, satu kes yang menunjukkan histologi DN memaparkan imunonegativiti kepada YAP-1 dan beta catenin, dan tergolong kepada subkumpulan molekul bukan SHH/WNT. Majoriti (88.2%) medulloblastoma berada di lokasi pertengahan ventrikel keempat, termasuk varian DN. Dianggarkan tiga tahun kelangsungan hidup bebas penyakit (DFS) dan survival keseluruhan (OS) masing-masing adalah 60% dan 86.7%. Umur <3 tahun semasa diagnosis, ukuran tumor >5 cm, histologi LCA dan kumpulan berisiko tinggi adalah berkorelasi terbalik dengan DFS. Bayi <3 tahun mempunyai OS yang lebih teruk. Faktor-faktor lain tidak mempunyai kesan yang signifikan terhadap DFS dan OS. Kami telah membuktikan aplikasi imunohistokimia YAP-1 dan beta catenin ini berguna dan boleh dipercayai sebagai penanda surrogate dalam membantu mengkategorikan medulloblastoma kepada subkumpulan molekul yang berbeza. Nilai prognostik dan ramalan imunomarker YAP-1 dalam medulloblastoma pula

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hendaklah menunggu siasatan lanjut.

Kata kunci: beta catenin, ketumbuhan otak, medulloblastoma, ramalan, YAP-1

ABSTRACT

Four core molecular subgroups of medulloblastomas have recently been introduced disparate by their transcriptional profile, all of which have different prognostic value. We aimed to determine the histological variants and molecular subtypes of medulloblastomas by immunohistochemistry in our population, and to correlate that with clinicopathological parameters. Seventeen patients aged four-month to 14.3 years diagnosed with medulloblastoma were recruited from year 2002 to 2017. All medulloblastomas were assigned to various histological variants and molecular subgroups by immunohistochemistry surrogate markers (YAP-1 and beta catenin). They were then correlated with clinicopathological parameters and outcomes. Classic histology (76.5%) was the commonest, followed by large cell/anaplastic (LCA) (17.6%) and desmoplastic/nodular (DN) variants (59%). The most frequent molecular subgroup was non-SHH/WNT tumours (64.7%), seconded by SHH tumours (35.3%). Among the SHH tumours, 66.7% was classic histology and the remaining 33.3% was LCA variant. Interestingly, one DN histology demonstrated YAP-1 and beta catenin immunonegativity, denoting non-SHH/WNT molecular subgroup. Majority (88.2%) medulloblastomas were at midline 4th ventricle location, including DN variant. Estimated three-year disease-free-survival (DFS) and overall-survival (OS) was 60% and 86.7%, respectively. Age <3 years at diagnosis, tumour size >5 cm, LCA histology and high risk group were inversely correlated with DFS, with early relapse. Infant <3-year-old had worse OS. Other factors had no significant impact on DFS and OS. We had demonstrated the feasibility of simple immunohistochemistry-based surrogate markers (YAP-1 and beta catenin) to stratify medulloblastomas into distinct molecular subtypes. Prognostic and predictive values of YAP-1 immunomarker in medulloblastomas however await further investigations.

Keywords: beta catenin, brain neoplasms, medulloblastoma, prognosis, YAP-1

INTRODUCTION

Medulloblastoma is among the commonest malignant embryonal tumour of the cerebellum, accounting for approximately 25% of all paediatric intracranial neoplasms. Majority of cases are diagnosed in children less than 18 years of age, although rare occurrence in adults had been previously reported (Murase et al. 2018).

Four histologically defined subgroups of medulloblastoma were

recently described by the latest version of 2016 World Health Organisation (Pietsch et al. 2016) classification: classic, desmoplastic/nodular (DNMB), medulloblastoma with extensive nodularity (MBEN) and large cell/ anaplastic (LCA) medulloblastoma. Recent genomic studies have greatly enhanced our understanding of the intrinsic molecular biology of medulloblastoma, according to the 2016 WHO classification. Integrated genomic data revealed marked genetic heterogeneity within medulloblastoma, identified four molecularly defined subtypes; Wingless (WNT), Sonic Hedgehog (SHH), Group 3 and Group 4, with distinct gene expression profiles, demographics, biological behaviour and disparate clinical outcome (Taylor et al. 2012). An integrated diagnosis for medulloblastoma that requires additional molecular genetic information incorporated into traditional histological subtyping is currently advocated for routine practice (Northcott et al. 2012).

The traditional believe that this tumour arising from a single neural stem cell precursor of the external granule layer of the developing cerebellum is no longer holds true, following the identification of different medulloblastoma molecular subtypes (Ellison et al. 2011). It is now clear that WNT tumours originate from lower rhombic lip progenitor cells of the dorsal brainstem, while SHH tumours arise from cerebellar granule neuron precursors of the external granular layer. Ventricular zone stem cells are the presumed cells of origin for Group 3. Origin of Group 4 however remains uncertain, and is thought to originate from deep nuclei precursor cells in upper rhombic lip of cerebellar vermis (Juraschka & Taylor 2019).

Immunohistochemistry stains had been used as surrogate markers to categorise medulloblastoma into four distinct molecular subgroups. WNT-activated medulloblastoma is known to express β-catenin protein, while SHH-activated medulloblastoma expresses yes-associated protein-1 (YAP-1) and GRB2-associated binding-1 (GAB-1) proteins. Group 3/4 medulloblastoma are not known to express β-catenin, YAP-1 and GAB-1 proteins (Ellison et al. 2011).

β-catenin, a 92-kDa protein associated with E-cadherin, plays a crucial role in the WNT signal transduction pathway. It acts as a key transcriptional activator which stimulates cell proliferation. Dysregulation of β-catenin as demonstrated by nuclear expression of β-catenin immunohistochemically, is associated with carcinogenesis in medulloblastoma and many other cancers, including melanoma, lung, colorectal, endometrial and ovarian cancers (Morin 1999).

YAP-1, a transcriptional regulator within the nucleus, participates in promoting cell proliferation and inhibiting apoptosis. Nuclear translocation of YAP-1 is negatively regulated by the Hippo pathway upon activation by inducing phosphorylation (Fernandez et al. 2009). In medulloblastoma, it is recently hypothesised that SHH signalling interacts with the Hippo pathway and promotes nuclear localisation of YAP-

1 within cerebellar granule neuron which drives tumourigenesis (Ahmed et al. 2017; Fernandez et al. 2009).

The aetiology of medulloblastoma remained to be elucidated in most patients; although positive associations between parental occupation exposures to pesticide use, hydrocarbons, heavy metals and N-nitroso compounds with increases the risk of medulloblastoma have been previously postulated (McKean-Cowdin et al. 1998). Some inherited genetic conditions including Turcot and Gorlin syndromes predispose a child to certain molecular subgroups of medulloblastoma.

Turcot syndrome type II arises from mutation in adenomatous polyposis coli (*APC*) gene mutation, is strongly linked with medulloblastoma. *APC*, a tumour suppressor gene functions as a negative regulator of the WNT signalling pathway, by mediating degradation of β-catenin – a crucial transcription factor in cell proliferation (Hamilton et al. 1995). Inactivation of *APC* gene hence increases susceptibility to brain tumours. Besides, deregulation of the WNT pathway following somatic mutation of *CTNNB1* gene which encodes β-catenin protein initiates the development and progression of brain tumours, particularly WNT medulloblastoma (Zurawel et al. 1998; Northcott et al. 2012).

Recurrent somatic mutation of patched 1 (*PTCH1*) gene was reported in association with sporadic medulloblastoma, following the discovery of Gorlin syndrome (Johnson et al. 1996; Raffel et al. 1997). Gorlin syndrome is a rare, autosomal dominant genetic disorder, related to the mutation in *PTCH1* tumour suppressor gene – a key inhibitor of the Sonic Hedgehog (SHH) pathway. *PTCH1* germline mutation hence results in activation of the SHH signalling pathway, leading to uncontrolled cell proliferation and inhibition of apoptosis (Al-Rahawan et al. 2018).

Conventional treatment for medulloblastoma, which involves maximal surgical tumour removal followed by craniospinal irradiation and chemotherapy, predisposes a child at risk of long-term radiation-induced neurocognitive morbidity. Distinct biological properties among the medulloblastoma molecular subgroups allow reconsideration of craniospinal irradiation and treatment protocols tailored to the molecular status of the tumour (Thomas & Noel 2019), in the hope to minimise intervention-related long-term morbidity.

The aim of this study was to determine the histological variants of childhood medulloblastoma as well as their molecular subtypes by immunohistochemistry in our population and to correlate the distinct histological and molecular subtypes with their clinicopathological parameters including age, gender, ethnicity, histological subtypes, tumour stage, disease free survival and overall survival. To the best of our knowledge, this is the first being carried out in Malaysia.

MATERIALS AND METHODS

Study Population

Table 1: Primary antibodies

Antibody	Source	Concentration	Dilution	+ve Control
Beta catenin Code no: ab52771	Rabbit monoclonal (Abcam)	250 µg/ml	1:100	Breast cancer tissue
YAP-1 Code no: ab32572	Rabbit monoclonal (Abcam)	100 µg/ml	1:100	Breast cancer tissue

We conducted a retrospective, descriptive study using archival histopathological materials from Department of Pathology and Medical Records, Universiti Kebangssan Malaysia Medical Centre (UKMMC). All histologically diagnosed medulloblastoma cases from January 2002 to June 2017 were included in this study. Cases with insufficient clinical data and lost to follow up or paraffin-embedded tissue blocks that were not available; either lost, insufficient or destroyed and cases with equivocal features or indefinite diagnosis were excluded. All the relevant clinicopathological data, including age at diagnosis, ethnicity, histological diagnosis, tumour staging, treatment, clinical outcome and survival, were retrieved from the record office in UKMMC. Patients' identities were remained anonymous and each subject was coded accordingly.

Histopathological Evaluation

For the identified cases, all their tissue slides which had been stained with Haematoxylin and Eosin (H&E) were retrieved from the archive of the Department of Pathology and reviewed. Histological subtypes of medulloblastoma were carefully examined and assigned conforming to the 2016 WHO subcategorisation. Briefly, classical medulloblastoma, being the commonest histological subtype, comprise high grade small round blue malignant cells with high mitotic count and tumour necrosis. Homer-Wright rosettes are occasionally apparent. Large cell medulloblastoma was defined by monomorphic large round cells with prominent nucleoli while anaplastic medulloblastoma was marked by cytological anaplasia. These two entities are currently jointed under the umbrella group as LCA medulloblastoma. Desmoplasia/ nodular medulloblastoma, as its name implies, has reticulin-poor pale nodules with neurocytic differentiation surrounded by densely packed mitotically active malignant cells with intercellular desmoplasia, highlighted by additional reticulin preparations. MBEN, on the contrary, is a more mature version of DN medulloblastoma with larger and more pronounce reticulin-free nodules that are neuropils- and ganglion cell-rich, and sparse internodular desmoplasia.

Selection of Tissue Blocks and Antibodies

Besides, one slide with best representative of the lesion was chosen for each case. The corresponding block was retrieved for immunohistochemical staining. For each case, three new

sections were obtained from the formalin-fixed, paraffin-embedded block. Antibodies selection were based on a study by Ellison et al. (2011), as tabulated in Table 1. Normal colorectal tissue was used as positive control for β-catenin, while human breast carcinoma acted as positive control for YAP-1. Immunohistochemical staining, optimisation and verification of the immunostains were performed manually according to manufacturer's instructions.

Antibodies Staining Protocol

Immunohistochemical staining was carried out on an automated slide immunostainer (VENTANA BenchMark ULTRA, USA) using anti-β-catenin and anti-YAP-1 antibodies. Tissue blocks were cut at 3-micron thickness and mounted on positively-charged slides. The tissues were dewaxed in xylene, rehydrated and pre-treated by EnVision™ FLEX Target Retrieval Solution, High pH (Dako, Denmark) for 30 minutes at 110°C to enhance signal detection. This was followed by incubation with EnVision™ FLEX Peroxidase-Blocking Reagent (Dako, Denmark) for another 10 minutes. The tissues were then incubated with primary monoclonal antibodies at room temperature for 30 minutes, followed by incubation with EnVision™ FLEX HRP detection system (Dako Denmark) and DAB chromogen at room temperature for 30 and 10 minutes, respectively. Haematoxylin 2 (ThermoScientific, USA) was applied as counterstain. Sequential dehydration with xylene and alcohol at increasing concentration were performed and the slides were subsequently coverslipped, ready for interpretation.

Antibodies Staining Interpretation

Immunohistochemical analysis was performed by two pathologists blinded from the original histologic diagnosis. Whenever there was discordant result, the slides were reviewed together and a consensus was agreed upon. For β-catenin, only nuclear staining demonstrated in the tumour cells is considered positive, while YAP-1 positivity is defined by nuclear and/or cytoplasmic staining of tumour cells. For the two antibodies, strong staining of >10% of the tumour cells was considered as positive. Molecular subgrouping was identified based on the immunoexpression of surrogate markers i.e. WNT subgroup (β-catenin+), SHH subgroup (YAP-1+, β-catenin-) and non-WNT/SHH subgroup (YAP-1-, β-catenin-), including group 3 and group 4. In addition, the staining characteristics were scored for percentage of positive cells (0-100%) and intensity of staining (0-3+) semiquantitatively following which H-score was calculated (Fedchenko & Reifenrath 2014).

Risk Stratification

Medulloblastoma was clinically stratified the patients into average/standard risk and high risk according to Modified Chang system (Chang et al. 1969). High risk was defined as those patients who were under three years of age at diagnosis, have residual

disease (M+) or local residual disease above 1.5 cubic centimeters (cm) post operatively.

Data on treatments including surgical resection, craniospinal irradiation and high dose chemotherapy were collected. Follow-up data until death or their most recent available medical record data were documented. Disease free survival (DFS) was defined as the time completed treatment to the first detection of recurrence/drop metastasis or death; while overall survival (OS) was defined as the time from tumour diagnosis to last follow up/death. The data was analysed using Statistical Packaged for the Social Science (SPSS) software. The correlations between clinicopathological parameters and molecular subtypes of medulloblastoma were compared using a Chi-square test or Fisher exact test. Survival analysis was performed using the Kaplan Meier survival method. A p value of <0.05 was considered as significant.

RESULTS

Study Population

A total of 17 cases of medulloblastoma identified from January 2002 to June 2017 in our centre, which fulfilled the inclusion and exclusion criteria. The study population included all children and teenagers with mean age at diagnosis were 9 years (median 9 years 4 months, range 4 months - 14 years 4 months). None of them was adult. There was a preponderance of males in the study population, with a male to female ratio of approximately 2:1. In terms of ethnicity, most of the patients were Malays (n=14), followed by Indians (n=2) and Chinese (n=1). Of note, the majority of the cases arose in the midline of posterior fossa (with 12 cases from fourth ventricle and three

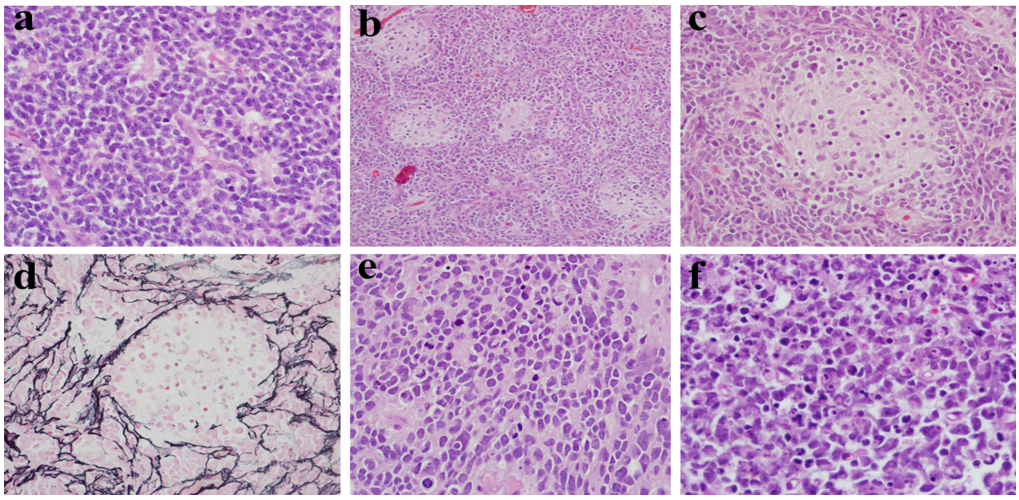

Figure 1: Histological variants of medulloblastomas. (a) Classic (H&E x400), (b-c) Desmoplastic/nodular (H&E x200, x400), (d) Desmoplastic/nodular (Retics x400) and (e-f) Large cell/anaplastic medulloblastomas (H&E x400).

Table 2: Correlation between histological subtypes with clinicopathological features in medulloblastomas

Parameters	Histological Subtypes			P value
	Classic n (%)	DN n (%)	LCA n (%)	
Age				0.235
<3 years old	0	0	1	
>3 years old	13	1	2	
Gender				0.341
Male	7	1	3	
Female	6	0	0	
Ethnicity				1.0
Malay	10	1	3	
Chinese	1	0	0	
Indian	2	0	0	
Tumour size (cm)	5.0±1.2	2.0	4.4±2.1	0.179
Location				0.426
Midline	12	1	2	
Non midline	1	0	1	
Mets at diagnosis				0.339
Yes	3	1	0	
No	10	0	3	
Risk stratification				1.0
Standard	6	0	1	
High	7	1	2	
Tumour staging				0.339
M0	10	0	3	
Any M	3	1	0	
Total	13	1	3	

DN – desmoplastic/nodular; LCA – large cell/anaplastic

from cerebellar vermis); while the remaining two cases were found in the cerebellar hemisphere.

Histological Subtypes

Three histological subtypes of medulloblastoma were identified in the study population (Figure 1). Classic variant was the commonest histological subtypes (n=13), contributing to 76.5% of all tumours with mean age at diagnosis was 9 years and 2 months (median 9 years, range 4 years - 14 years 4 months). LCA variant accounted for 17.6% (n=3) of all tumours, found in a four-month-old infant, ten- and eleven-year-old boys. DN variant was the least common subtype (n=1) in the cohort, occurring in a 12-year-old boy.

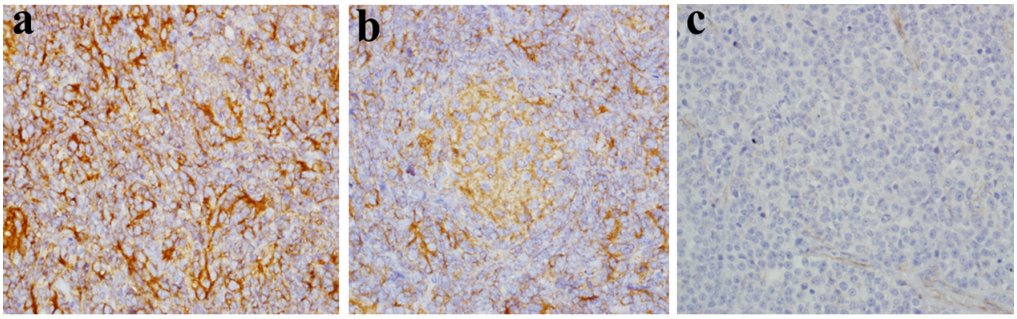

Figure 2: Immunohistochemistry Staining Pattern with Beta Catenin. Tumour cells in (a) classic histology (x400) and (b) desmoplastic/nodular variant (x400) reveal membrane immunopositivity and (c) complete immunonegativity (x400), indicating non-WNT tumour.

Comparing the location of various histological subtypes, it was observed that majority (92.3%) of classic variant was found in the midline fourth ventricle including three from cerebellar vermis, while only one (7.7%) were laterally located within the cerebellar hemisphere. All the LCA variants were found in the midline fourth ventricle. Interestingly, the DN variant displayed a midline location at the roof of fourth ventricle.

The mean size of tumour for classic variant was 5.0±1.2 cm, relatively larger than LCA and DN variants with 4.4±2.1 cm and 2.0 cm, respectively, although not statistically significant ($p = 0.179$). Details on demographics and clinicopathological characteristics of the three histological subgroups were illustrated in Table 2.

Debulking surgeries were performed on almost all patients (94.1%) except one patient with DN variant presented with inoperable tumour with extensive metastasis at time of diagnosis. Almost all patients (92.8%) with classic variants received craniospinal radiotherapy and completed chemotherapy, with the exception of one patient whose parents refused chemoradiotherapy after debulking surgery and died. One infant with LCA variant died two months after surgery with tumour progression complicated by intraventricular bleed.

WNT Medulloblastomas

All 17 cases that were analysed by immunohistochemistry for β-catenin immunoexpression revealed negative reaction, indicated non-WNT tumours. None of the cases showed positive nuclear staining against β-catenin, while cytoplasmic positivity was seen in nine cases and eight cases revealed no staining at all (Figure 2).

SHH Medulloblastomas

SHH medulloblastomas were identified by their nuclear and cytoplasmic YAP-1 immunoreactivity but lacking the expression for β-catenin. Details on demographics and clinicopathological characteristics of the SHH and non-SHH/WNT subgroups were summarised in Table 3. SHH tumours constituted six (35.3%) of all medulloblastomas in the present study,

Table 3: Correlation of YAP-1 immunoexpression with clinicopathological features in medulloblastomas

Parameters	YAP-1 Immunoreactivity		P value
	Positive n (%)	Negative n (%)	
Age			0.005*
<3 years old	1	0	
3-12 years old	1	10	
> 12 years old	4	1	
Gender			1.0
Male	4	7	
Female	2	4	
Ethnicity			0.272
Malay	4	10	
Chinese	1	0	
Indian	1	1	
Tumour size (cm)	5.2±1.0	4.4±1.6	0.369
Location			1.0
Midline	5	10	
Non midline	1	1	
Mets at diagnosis			1.0
Yes	1	3	
No	5	8	
Histological subtypes			0.688
Classic	4	9	
DN	0	1	
LCA	2	1	
Risk stratification			1.0
Standard	2	5	
High	4	6	
Tumour Staging			1.0
M0	5	8	
Any M	1	3	
Total	6	11	

DN – desmoplastic/nodular; LCA – large cell/anaplastic
*statistically significant

with predominantly classic variant (66.7%), followed by LCA variant (33.3%). All SHH tumours exhibited strong (3+) to moderate (2+) nuclear and cytoplasmic staining intensity in at least 20% of tumour areas (Figure 3), with H-score range 120-285. SHH tumours were positively correlated

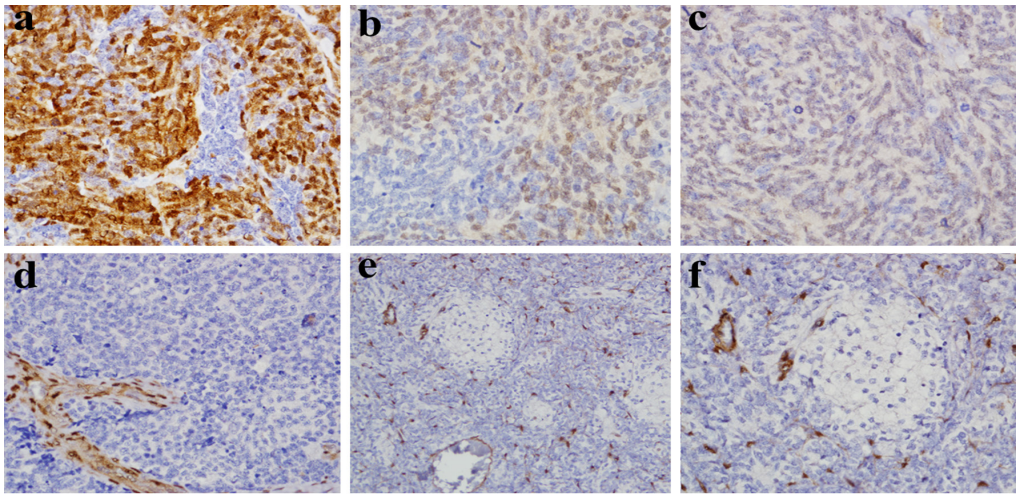

Figure 3: Immunohistochemistry Staining Pattern with YAP-1. Tumour cells exhibit YAP-1 nuclear and membrane immunoreactivity (x400) at (a) 3+, (b) 2+ and (c) 1+, conclude a SHH-activated tumour, while (d) complete immunonegativity (x400), consistent with non-SHH/WNT tumour; (e-f) One desmoplastic/nodular medulloblastoma exhibits YAP-1 negativity (x200, x400).

with age at diagnosis, in which there were two age peaks observed, i.e. the first in the first three years of life and the second occurred after the age of 12 (p=0.005). We found that there was no gender preponderance with approximately 1:1 male to female ratio among SHH medulloblastomas.

Majority (83.3%) SHH tumours were found located within the midline fourth ventricle, while the remaining one located peripherally within cerebellar hemisphere. Of note, SHH tumours were relatively larger than non-SHH/WNT subtypes with mean tumour size 5.2±1.0 cm, although not proven statistically significant. Except for one patient, none of the patients with SHH tumours presented with metastases at time of diagnosis.

Non-SHH/WNT Medulloblastomas

A total of 11 (64.7%) non-SHH/WNT tumours as indicated by negative YAP-1 and β-catenin immunoreaction were identified. Classic variant constituted 81.8% of non-SHH/WNT tumours, followed by DN (9.1%) and LCA (9.1%) variants. Intriguingly, none of the DN variant demonstrated YAP-1 immunoreactivity. Non-SHH/WNT tumours, unlike SHH tumours, occurred frequently in children age between three to 12 years (p=0.005), with slight male preponderance. Nearly all (90.9%) non-SHH/WNT tumours arose from midline fourth ventricle, with mean tumour size was 4.4±1.6 cm (range: 3.8-6.5 cm). About 37.5% of non-SHH/WNT tumours presented with metastasis at time of diagnosis.

Survival Analysis

In this study, there were two patients who defaulted follow-up prior to

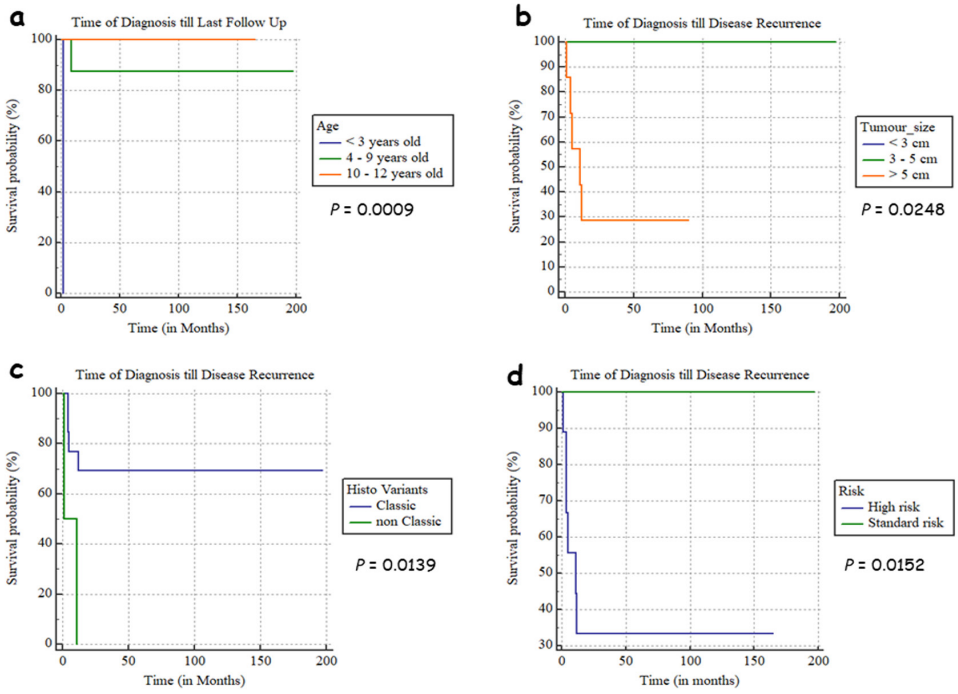

Figure 4: Kaplan Meier analysis of Disease-free-survival according to (a) Age at diagnosis, (b) Tumour size, (c) Histological variants and (d) Risk stratification.

completion of treatment. The mean follow-up time was 82.1 months (range: 2-197 months). The overall median survival time of medulloblastoma was 77 months (95% CI 54.9 - 109). The three-year disease-free survival (DFS) and overall survival (OS) rate was 60% and 86.7%, respectively.

Classic histology showed a significant better outcome with median DFS 67 months compared to LCA histology with median DFS six months (p=0.0139). Expectedly, the high risk group and those with tumour size more than 5 cm at initial presentation had worse DFS with early relapse compared to those in the standard risk category (p=0.0152) and with smaller tumour size at diagnosis (p=0.0248). The prognosis was grim for infants less than 3 years old with poor OS (p=0.0009) and DFS (p=0.0008) (Figure 4). Other factors including gender, tumour location, YAP-1 expression H-score and molecular subtypes did not affect DFS in our cohort. Similarly, there were no significant differences in OS for gender, size of tumour, tumour location, tumour staging, histological variants, YAP-1 expression H-score and molecular subtypes.

DISCUSSION

Medulloblastoma accounts for about 20% of childhood brain tumours globally, being the second commonest after haematological malignancies in paediatric population. Overall incidence of medulloblastoma is

low (0.49/100,000 children/year) worldwide (de Robles et al. 2015). At our centre, only 17 cases of medulloblastomas were diagnosed in the 15-year study period. This is slightly lower than that found in Hospital Kuala Lumpur, a tertiary neurosurgery referral centre in Malaysia with 66 reported cases in 20 years (Shaalih et al. 2018).

Medulloblastomas occur in patients of all ages, with peak incidence in children age between 4 and 9 years of age, followed by adolescents (10 to 16 years old) and infants/toddlers (0-3 years old). Our patients were slightly older than those reported in the literature; with the median age at diagnosis was 9 years. Males were two times more often affected than females, in accordance to most literature (Smoll & Drummond 2012).

In this study, we attempted to stratify our medulloblastoma cases into various histological subtypes and distinct molecular subgroups according to their immunoreactivity to surrogates immunomarkers. Due to lack of infant age group diagnosed with medulloblastomas in our study population, the so-called infant-exclusive MBEN variant was not detected in the current study. Three distinct histological variants had been identified with frequency 76.5%, 17.6% and 5.9% for classic, LCA and DN variants, concurred with Schroeder & Gururangan (2014).

Two molecular subtypes (SHH and non-SHH/WNT) of medulloblastomas were demonstrated by immunohistochemistry. Immunohistochemistry was proven to be a reliable surrogate to define a precise subgroup. WNT-activated medulloblastomas accounted for about 10% in many international studies (Cambruzzi 2018). Regrettably, no WNT subgroup was identified in our study population.

SHH-activated tumours constituted about 35% of all medulloblastomas in this study, in agreement to Miranda et al. (2018). On the contrary, we observed that as high as 80% SHH-activated tumours located in the midline rather than laterally in the cerebellar hemispheres, as reported in many studies. This may be attributed to the small sample size in our cohort and may not be representative of the entire population.

In agreement to Kool et al. (2012), this SHH tumours had a bimodal age distribution, which affected infants below the age of three years as well as older children (more than 12 years old), with no gender preponderance. Approximately 20% of SHH-activated medulloblastomas presented with metastasis at the time of diagnosis. There had been a dispute in regards to the outcomes of SHH subgroup. Cohen et al. (2015) in their pilot study reported an excellent prognosis in infant DN and MBEN tumours, while dismal prognosis in this subgroup of paediatric patients was also previously described. Other prognostic factors include TP53 and PTCH1 that might have survival impact in this subgroup had been proposed.

Although DN variant and MBEN are found typically associated with SHH subgroup, more than half of the SHH tumours are of classic histology and rarely LCA histology (Pietsch et

al. 2016). Intriguingly, we observed that not all DN variant should fall under the SHH category. One of our cases that was proven DN variant histologically, revealed YAP-1 and β-catenin immunonegativity consistent with non-SHH/WNT subgroup. However, this observation required further validation.

Non SHH/WNT subgroup was identified in about 60% of all medulloblastomas, in agreement with the findings of many studies (Schroeder & Gururangan 2014), which carried a poorer prognosis. We observed that as high as 40% of these patients had spinal metastases at time of diagnosis.

Our prognostic indices were comparable with previous studies. Prior to the molecular era, overall 3- and 5-year survival risk for medulloblastomas was 66% and 55% respectively (Igual Estellés et al. 2017). Our data showed a slight better 3-year OS rate of 87.6%. We reported a 100% and 75% 3-year OS among the standard and high risk groups, respectively, somewhat higher than that reported by others (70-80% for standard risk disease and 60-65% for high risk disease). LCA histological variant especially had shown to affect progress-free survival rate, with higher risk of relapse, parallel to previous studies (Igual Estellés et al. 2017). Age too was one of the significant predictors, for those age >3 years which had a better OS and DFS than patients age <3 years (Juraschka & Taylor 2019).

Many studies focused mainly on the residual tumour size post operatively, with very few investigated on the impact of tumour size on survival. Although tumour size at diagnosis was not found to affect OS, and we noticed that the tumour size of more than 5 cm, had a significantly worse DFS than those with smaller tumours, with early progression of tumour. In contrast to this, Nalita et al. (2018) reported no significant survival benefits for those with smaller tumour at diagnosis.

YAP-1 is identified as an oncogene in various human malignancies, which influences carcinogenesis and progression of cancers. Elevated YAP-1 protein expression was reported to be correlated with inferior OS and worse outcomes in many carcinomas including oral, gastrointestinal, hepatocellular as well as non-small cell lung cancers (Su et al. 2012; Zhang et al. 2018). Besides utilising YAP-1 as surrogate marker for SHH subgroup, in the current study, we attempted semiquantitative immunohistochemistry analyses approach on YAP-1, in the hope to recognise a better categorisation of medulloblastomas than a mere positive or negative results towards improved therapeutic decisions. High expression of YAP-1 had no prognostic implication in our limited study population.

This study nonetheless is subjected to limitation. The small sample size recruited from our study population had restricted our ability to detect significant correlations between the medulloblastoma phenotypes/genotypes with some clinicopathological parameters and survival. Despite the limitation, our study provided some evidence that not all histological DN variant was

associated with SHH molecular subgroup, a finding that had rarely been reported previously. Future large scale and well-designed studies are warranted to validate these findings.

CONCLUSION

In summary, we have demonstrated the feasibility of simple immunohistochemistry-based surrogate markers (YAP-1 and β-catenin) to stratify medulloblastomas into distinct molecular subtypes. Prognostic and predictive values of YAP-1 immunomarker in medulloblastomas require further investigations.

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