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Original Research

Clinicopathological Characteristics of non-WNT/non-SHH Medulloblastoma Cases in a Pediatric Egyptian Cohort

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Objective: Medulloblastoma is the most common malignant pediatric brain tumor. It has a great impact on global health. Although, current treatment modalities improve patients` survival rates, survivors suffer from long term treatment related morbidity. Major advances have changed molecular understanding of medulloblastoma with the emergence of the molecular classification of medulloblastoma that has been introduced in WHO classification of CNS tumors. Non-WNT/non-SHH molecular subgroup is the most common molecular subgroup representing more than 60% of medulloblastoma cases. The present study aims to describe the clinical, pathological, and survival characteristics of pediatric patients with non-WNT/non-SHH MB.
Results: A total of 36 non-WNT/non-SHH MB cases were detected. The age of the patients ranged between 2 to 18 years. 14 patients (39%) were standard risk while 22 patients (61%) were high risk. Microscopic evaluation showed that 34 cases (94.4%) were of classic histology, while 2 cases (5.6%) were of LC/A histology. The 5-year overall survival of the 36 non-WNT/non-SHH cases detected was 55% and the 5-year event free survival was 40%.
Conclusion: The clinical, pathological, and molecular characteristics of pediatric patients with non-WNT/non-SHH molecular subgroup of medulloblastoma described in the present study were mostly similar to those reported in the literature.

Introduction

Brain tumors are the leading cause of cancer death in children, with medulloblastoma (MB) being the most common malignant pediatric brain tumor representing 20% of pediatric brain cancers [1]. Unfortunately, disease dissemination is an early event, and as many as 40% of patients show metastases at the time of diagnosis [2]. Current multimodal treatment approaches cure approximately 60% to 75% of patients. Over the past decades, metastatic disease and tumor recurrence are responsible for the poor survival rates, while survivors reveal impaired neurologic function, endocrine dysfunction, and cognitive sequelae secondary to surgical resection, irradiation, and chemotherapy [3].

MB proved through genome-wide analysis to be a very heterogenous disease and are divided into four molecular subgroups. The current consensus identifies four distinct molecular entities within MBs: wingless-activated (WNT), sonic hedgehog-activated (SHH), group 3, and group 4 MBs. The provisional non-WNT/non-SHH entity combining group 3 and group 4 represents over two thirds of all MBs, together with the highest rates of metastases and least understood pathology [4]. The

demographic, transcriptional, genetic, and clinical differences between these four groups are pronounced. Additionally, molecular classification is prognostic with markedly different survival rates. This prognostic significance may play a role in improving the treatment of patients with MB [5].

At present, treatment intensity for MB is risk stratified on the basis of clinicopathological biomarkers such as age at presentation, extent of resection and the presence of metastases, as well as the pathological diagnosis. However, clinicopathologic features often fail to accurately predict treatment response [6]. Therefore, risk assessment guided by molecular classification has become an emerging necessity to improve outcome of high-risk patients and to decrease treatment-related toxicity and long-term sequelae in standard-risk patients [7].

WNT molecular subgroup account for 10-15% of MB patients with excellent survival rates. They do not present in infants while they have the tendency to present in at older age than that for all pediatric MBs. These tumors are defined by mutations related to the respective pathway, such as CTNNB1 or APC [8].

SHH MB represent 25-30% of all cases. They are well presented in infancy and tend to show intermediate risk. mutations and copy number variations (CNVs) of SHH-pathway members and alterations of TP53 and TERT are characteristic genetic hallmarks [9].

Group 3 and group 4 molecular subgroups are more related to each other than to WNT and SHH and appear as non-WNT/non-SHH in the revised 2016 WHO classification [10]. Non-WNT/non-SHH MB is the most common molecular subgroup representing 60% of all cases and remains the genetically most heterogeneous and least understood fraction of MB cases. There is no single defined mutation could be detected in more than 10% of the cases [11].

Recently, a combined genome-wide DNA copy-number and mRNA expression analysis was used to define a classification system based on immunohistochemistry (IHC). This strategy identifies reliable IHC markers to designate distinct, nonoverlapping molecular subgroups of MB; WNT, SHH and non-WNT/non-SHH [12].

The introduction of the new WHO classification, as well as the stratification and new therapeutic protocols based on molecular data, will guide more effective approaches to improve MB treatment. To that end, we must evaluate the current results of pediatric patients treated in an Egyptian center specialized in pediatric oncology. The present study aims to describe the clinical, pathological, and survival characteristics of pediatric patients with non-WNT/non-SHH MB.

Materials and Methods

Retrospective analysis of pediatric MB patients treated at Borg El Arab oncology center, Egypt over 10-year-period. Formalin-fixed paraffin-embedded (FFPE) blocks were retrieved from pathology department, Faculty of Medicine, Alexandria University, Egypt, while complete clinical and survival data were collected from oncology records at Borg El-Arab oncology center, Egypt. The current study was conducted after the approval of research ethics committee, Alexandria University.

Histopathologic examination

MB cases were classified into three histologic variants:

classic, D/N and LCA following 2021 WHO classification of CNS tumors [13].

Immunohistochemical analysis

Molecular subgrouping of MB: SHH, WNT, and non-WNT/non-SHH subgroups was done by IHC using a combination of three antibodies; β-catenin antibody, GAB1 antibody, and YAP1 antibody [14, 15]. The non-WNT/non-SHH cases were chosen to be included in the study.

FFPE tissue blocks were cut into 4-µm sections. Deparaffinization and antigen retrieval were performed in a Dako PT Link unit. Both high and low pH EnVision TM FLEX Target Retrieval Solutions were used at 97 °C for 20 minutes. Dako autostainer (Link 48, Agilent Technologies, Inc, CA, USA) was used for immunostaining.

Risk stratification

Patients were risk stratified into standard risk and high risk based on age at diagnosis, postoperative residual mass, histological variant, and leptomeningeal dissemination. Patients were considered standard risk when they are >3 years of age, absence of LCA histology, no post operative residual mass or post operative residual mass under 1.5 cm^2 and without leptomeningeal dissemination [16]. Statistical analysis: Statistical analysis was performed using the IBM Statistical Package for Social Sciences (SPSS) version 25 (Chicago, IL, USA) [17]. Data were expressed as frequencies for categorical variables, and continuous variables were expressed as mean (M) ± standard deviation (SD) or median (Mdn) and range.

Survival analyses [overall survival (OS) and event free survival (EFS)] were performed. OS was the time from date of diagnosis to death or the date of last follow-up. The event is either recurrence/progression or death, and EFS was calculated from date of end of treatment till date of event, or last visit. Log rank test was used to compare the patients' outcome between non-WNT/non-SHH molecular subgroup and SHH MB cases detected during immunohistochemical analysis for comparison purposes.

In all statistical tests, P value < 0.05 was considered statistically significant.

Results

Molecular classification

Based on IHC results, among all the specimens examined, non-WNT/non-SHH molecular subgroup (nuclear β-catenin negativity, cytoplasmic GAB1 negativity and nuclear YAP1 negativity) represented 36 cases (Figure 1).

Figure 1. Immunohistochemical Results Showing the Staining Pattern in Non-WNT/Non-SHH, Molecular Subgroup, (A) β-catenin Nuclear Negativity (X400), (B) GAB1 Cytoplasmic Negativity (X400), (C) YAP1 Nuclear Negativity (X200).

Patient characteristics

A total of 36 non-WNT/non-SHH MB cases were detected. The age of the patients ranged between 2 to 18 years with four patients ≤ 3 years of age (11.1%) and 32 > 3 years of age (88.9%). 22 patients were males (61%) while 14 were females (39%).

Clinical characteristics

The preoperative size was estimated as the maximum cross-sectional area on computerized tomography (CT) and ranged between 9 and 45 cm^2 with a mean of 16 cm^2. 11 cases (30.6%) showed leptomeningeal dissemination at the time of diagnosis while 25 (69.4%) patients did not

have leptomeningeal dissemination at the time of diagnosis. Post operative residual mass was assessed and showed that 22 (62%) of the patients did not have post residual mass, 4 (12%) patients had a residual mass <1.5 cm^2, while 10 (23%) showed >1.5 cm^2 post operative residual mass. When risk stratified, 14 patients (39%) were standard risk while 22 patients (61%) were high risk. On follow up, treatment response was assessed, and it was found that 31 (86.1%) showed either complete or partial response and 5 (13.9%) patients did not respond to treatment or progressed through the course of treatment.

Histopathological examination

Based on 2021 WHO classification of CNS tumors, microscopic evaluation showed 34 cases (94.4%) were of classic histology, while 2 cases (5.6%) were of LCA histology. Figure 2 and Table 1.

Figure 2. Histopathological Variants of Non-WNT/ Non-SHH MB (A), Classic Histology (H&E X100), (B) Large cell/anaplastic Histology (H&E X400).

	No. (%)
Age in years	
≤ 3	4 (11.1)
> 3	32 (88.9)
Sex	
Male	22 (61)
Female	14 (39)
Preoperative size cm^2	16 (9-45)
Leptomeningeal dissemination	
Yes	11 (30.6)
No	25 (69.4)
Post operative residual mass	
No	22 (62)
<1.5 cm^2	4 (12)
>1.5 cm^2	10 (23)
Risk stratification	
High risk	22 (61)
Standard risk	14 (39)
Pathology	
Classic histology	34 (94.4)
LC/A histology	2 (5.6)
Response to therapy	
Stationary& progressive course	5 (13.9)
Complete& partial response	31 (86.1)

Table 1. The Clinicopathological Features of the Studied non-WNT/non-SHH MB Patients.

(n=36)

Survival analysis

The 5-year overall survival (OS) of the 36 non-WNT/ non-SHH cases detected was 55% with a mean of 75.8 months and a median of 69 months. The 5-year event free survival (EFS) was 40% with a mean of 61.6 months and a median of 33 months. Although the OS and EFS of non-WNT/non-SHH

MB cases (55% and 40%) were lower than those of SHH molecular subgroup (70% and 42%), the difference was not significant (p= .862 and .678 respectively). Table 2 and Figure 3.

Molecular group		OS				EFS		
	Number of events	Meana [a]	Median	5-year %	Number of events	Meana [a]	Median	5-year %
non-WNT/non-SHH	17	75.847	69	55	21	61.611	33	40
SHH	6	73.846	66	70	7	64.754	56	42

Table 2. Relation of Overall Survival (OS) and Event Free Survival (EFS) between Non-WNT/Non-SHH and SHH Molecular Subgroups.

a. Estimation is limited to the largest survival time if it is censored

Figure 3. Kaplan-Meier Curves (A) Overall Survival (OS) in Relation to Molecular Subgroups (non-WNT/non-SHH and SHH), (B) Event Free Survival (EFS) in Relation to Molecular Subgroups (non-WNT/non-SHH and SHH).

Discussion

MB is the most common pediatric CNS tumor with global impact on children's overall health. MB proved to be heterogeneous disease. Even in patients with similar clinicopathologic features, the outcome may differ significantly. Molecular designation added prognostic value with distinctive clinicopathologic profile of each molecular subgroup [18].

Non-WNT/non-SHH molecular subgroup comprising group 3 and group 4 MB represent the most molecular subgroup which accounts for nearly 60% of all patients and remains the genetically most heterogeneous and least understood fraction of MB cases [19].

In the present work, non-WNT/non-SHH MB cases under the study presented in both infants and older children with most of the cases being more than 3 years of age (88.9%). Kool et. al [11] reported in their study that non-WNT/non-SHH molecular subgroup of MB could happen in both age groups. Northcott et. al [4] reported similar results to our study with this molecular subgroup being mostly represented in children between the ages of 3 and 18 years old. Eid and Heabah [20] found similar results in their study where 75% of non-WNT/non-SHH MB cases were above 3 years of age. Yehia et. al [21] also reported in their study comparable demographic characteristics with 95.16% of the patients were more than 3 years of age. Most of the patients under the current study were males (61%) similar to results reported by Eid and Heabah [20] where 66.7% of non-WNT/non-SHH MB patients were males. In the present study, most of the patients did not show leptomeningeal dissemination at the time of the diagnosis (69.4%) with only 30.6% of the cases showed leptomeningeal dissemination. Findings in the literature were quite different as non-WNT/non-SHH MB patients in the literature showed higher rates of metastasis at diagnosis. Ramaswamy et. al [22] reported rate of metastasis of 45% in group 3 and 40% in group 4. Yehia et. al [21] found that 48.3% of non-WNT/non-SHH MB patients presented by leptomeningeal dissemination. Aras et. al [23] also reported that 75% of non-WNT/non-SHH patients presented by metastasis. Similar findings were reported by Remke et. al. [24].

In the current work, the large majority of the patients (62%) underwent gross total resection. Higher rates of gross total resection were reported in the literature. Yehia et. al [21] reported 80.6% of gross total resection in non-WNT/non-SHH molecular subgroup. 92% of non-WNT/non-SHH MB patients included in HIT-SIOP PNET 4 clinical trial underwent gross total resection [25].

In the current study, most of the patients under the study were high risk representing 61% of the whole group. This is consistent with the findings reported in the literature and the fact that this molecular subgroup of MB is biologically associated with worse prognosis. Aras et. al [23] reported that 71% of non-WNT/non-SHH MB patients in their study were of high risk. Yehia et. al [21] also found that most of the patients belonging to non-WNT/non-SHH molecular subgroup were high risk representing 72.88%. Eid and Heabah [20] reported a rate of 83.3% of high risk patients in non-WNT/ non-SHH molecular subgroup.

In the present work, the histology of most of non-WNT/non-SHH MB cases showed classic histology (94.4) with only two cases (5.6%) showed LC/A histology. Similar findings were reported in the literature. Northcott et. al [4] reported that majority of cases belonging to group 3 and group 4 MB cases were of classic histology with minority of cases showing LC/A histology. Same results were reported by Remke et. al [24]. Aras et. al [23] also reported similar findings where 87.5% of non-WNT/ non-SHH MB cases showed classic histology while the rest showed LC/A histology. Eid and Heabah [20] found that higher rates of LC/A histology were represented in non-WNT/non-SHH molecular subgroup forming the majority of the group (54.2%) while the rest of the cases (45.8%) showed classic histology. Yehia et. al [20] reported also higher rate of LC/A histology within non-WNT/non-SHH molecular subgroup representing 35.48% of the group with the rest of the cases showing classic histology.

The 5-year OS of the patients under the current study was 55% while the EFS was 40%. Eid and Heabah [20] reported lower rates of survival for non-WNT/non-SHH MB patients with 33.3% OS at the end of the study and 12.5% progression free survival (PFS) at the end of the study. Aras et. al [23] reported higher survival rates with 81.6% 5-year OS and 51.8% 5-year EFS.

In the present work, patients in non-WNT/non-SHH molecular subgroup had lower 5-year OS and EFS than patients belonging to SHH molecular subgroup that were detected in the same period of analysis. However, there was no significant difference between the survival of both groups. Eid and Heabah [20] reported similar results with lower OS and PFS for non-WNT/non-SHH MB molecular subgroup than SHH molecular subgroup. They also found that the difference was significant. Remke et. al [24] reported comparable results with lower OS and PFS in non-WNT/non-SHH molecular subgroup than in SHH molecular subgroup. Yehia et. al [21] also found significant association between molecular subgroups and OS and EFS with non-WNT/non-SHH molecular subgroup showing the worst prognosis in all molecular subgroups. Aras et. al [23] found different results with higher survival rates for non-WNT/non-SHH molecular subgroup than other molecular subgroups. They also reported that there was no statistically significant difference among OS and EFS of molecular subgroups.

The clinical, pathological, and molecular characteristics of pediatric patients with non-WNT/non-SHH molecular subgroup of medulloblastoma described in the present study were mostly similar to those reported in the literature. This information and the continuous search for validation of global evidence with respect to local problems enables the participation of Egyptian facilities in international multicenter protocols for the treatment of medulloblastoma, which can significantly improve the clinical outcomes achieved.

The systematic knowledge of the molecular biology of medulloblastoma is crucial because it will allow the emergence of new specific therapeutic modalities focused on molecular targets, aiming at increasing survival and reducing treatment-related morbidities.

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