Brief Communication

Spectrum of medulloblastoma subtypes and frequency of MYC amplification; Experience from a tertiary care center in Saudi Arabia

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ABSTRACT

Objectives: To clarify the spectrum of morphological and molecular subtypes of medulloblastoma (MBL), in addition to MYC and MYCN amplification statuses in a cohort of Saudi patients. The latter was correlated with patient outcome.

Methods: We conducted a retrospective cohort study of 57 patients with MBL, diagnosed at the central laboratory of King Abdulaziz Medical City in Riyadh, Saudi Arabia, between 2006 and 2019. Molecular analysis for MYC and MYCN amplification was performed for the 19 most recently diagnosed patients.

Results: Classic MBL was the most prevalent histologic subtype and MBL with extensive nodularity was the rarest. The non-WNT/non-SHH molecular subgroup was the most common while the WNT-activated was the least common. Among 19 patients analyzed, MYC and MYCN amplifications were discovered in 2 (10.5%) and 1 (5.3%) cases, respectively, using interphase fluorescence in-situ hybridization. The 2 MYC amplified cases belonged to the large cell/anaplastic subtype and had the worst outcomes.

Conclusion: The MYC amplification corresponded with poor prognosis, the large cell/anaplastic variant of MBL, and the non-WNT/non-SHH molecular subtype.

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Medulloblastoma (MBL) is the most prevalent pediatric embryonal brain neoplasm originating from the cerebellum and dorsal brain stem.1 There are 4 histomorphological variants of MBL: classic, desmoplastic/nodular, large cell/anaplastic, and MBL with extensive nodularity.1-3 The MBLs are now classified into 4 molecular subtypes: Wingless (WNT)-activated, sonic hedgehog (SHH)-activated, group 3, and group 4. Groups 3 and 4 are less well defined from a molecular standpoint, and are difficult to distinguish from each other using commercially available surrogate biomarkers.4 For practical purposes, MBLs are therefore subdivided into 3 molecular subgroups: WNT-activated, SHH-activated, and non-WNT/non-SHH. Traditionally, patients with MBL were stratified according to their risk of recurrence (average risk: >3 years of age, no/minimal residual disease post-surgery, and no CNS metastasis; and high risk: <3 years of age exclusive to the extensively nodular subtype, significant residual disease post-surgery, and evidence of metastasis within the CNS).4

The MYC amplifications have long been associated with highly aggressive MBLs, and occur in 5% to 10% of MBLs.5 The purpose of this study was to recognize the spectrum of MBL histologic and molecular subtypes seen at our institute and to estimate the frequency of MYC and MYCN amplification. Additionally, the prognosis of the MYC amplified cases was compared to that of their non-amplified counterparts.

Methods. This was a retrospective cohort study carried out in the Department of Pathology and Laboratory Medicine at King Abdulaziz Medical City, Riyadh, Kingdom of Saudi Arabia. We reviewed the pathology reports, microscopic slides, and electronic medical files of Saudi MBL patients diagnosed between 2006 and 2019. A consecutive sampling technique was used and all patients’ unique identifiers were removed. All included MBL cases were reviewed independently by 2 certified neuropathologists (AHA and FMA) for morphological and molecular classification. The 19 most recent cases were analyzed for MYC and MYCN amplification as well monosomy 6, by the interphase fluorescent in-situ hybridization technique (iFISH). The latter was performed at the Mayo Clinic Laboratories. We prepared 10 unstained slides from tumor tissue paraffin blocks and mailed them abroad to the Mayo Clinic Laboratories for FISH analysis (https://www.mayocliniclabs.com/test-catalog/TestID: MEDF). This test uses commercially available probes designed to target MYC and MYCN foci while monosomy 6 is detected using the MYB probe. Additionally, immunostaining for beta-catenin, Yes-Associated Protein (YAP1) and GRB2-associated Binding Protein 1 (GAB1) was used to aid in molecular classification. YAP1 and GAB1 immunostaining was also performed at the Mayo Clinic Laboratories. The WNT-activated subgroup was identified by positive nuclear staining for beta-catenin.

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beta catenin, monosomy 6, and positivity for YAP1. The SHH-activated subgroup was identified by the presence of a nodular/desmoplastic pattern and/or positive staining for YAP1 and GAB1. The non-WNT/non-SHH subgroup was identified by a lack of evidence for WNT or SHH activation.

The inclusion criteria were as follows: Saudi patients diagnosed with MBL, available pathology reports, glass slide-mounted tumor samples, and available patients' medical records. Two out of 59 patients were excluded after applying these criteria.

The data were collected in Microsoft Excel (Microsoft Corp., Redmond, WA), and statistically analyzed using the software SPSS, version 20 (IBM Corp., Armonk, New York, USA). Categorical variables were measured as percentages and frequencies. Patient-specific characteristics included age, gender, and MYC/MYCN statuses. Cases were grouped according to their histologic subtype (classic, MBL with extensive nodularity, desmoplastic/nodular, and large cell/anaplastic) and molecular subtype (WNT-activated, SHH-activated, and non-WNT/non-SHH). Measured outcomes included metastasis, recurrence, and mortality. Kaplan-Meier survival curves were constructed to determine the survival rates as a function of MYC and MYCN amplification status.

This study was approved by the Institutional Review Board (IRB) of King Abdullah International Medical Research Center (RC17/105/R).

**Results.** Fifty-seven patients were included in the study; female patients had a slight majority (n=30, 52.6%). The patients' ages ranged from 1 to 48 years. Half of the patients were in the pediatric age group (defined as ≤14 years; n=29, 50.9%) as shown in Table 1. The non-WNT/non-SHH molecular subgroup was the most frequently identified (n=12; 63.3%). The majority of our MBL cases were of the classic subtype (n=39; 68.4%) while MBLs with extensive nodularity were the least prevalent (n=2; 3.5%).

Table 2 shows the distribution of histological MBL subtypes in relation to the statuses of MYC and MYCN amplification as well as patient outcomes. Only 2 out of the 19 tested cases were MYC amplified (10.5%); both were of the large cell/anaplastic subtype. MYCN amplification was identified in 1 out of the 19 tested

### Table 1 - Demographics and clinicopathological data. N=57

| Demographics       | Total n (%) |
|--------------------|-------------|
| Sex                |             |
| Male               | 27 (47.4)   |
| Female             | 30 (52.6)   |
| Age                |             |
| ≤14 years          | 29 (50.9)   |
| >14 years          | 28 (49.1)   |
| Histology Subtypes |             |
| Classic            | 39 (68.4)   |
| Desmoplastic/nodular| 12 (21.1)  |
| Anaplastic/large-cell| 4 (7.2)    |
| With extensive nodularity| 2 (3.5) |
| Molecular Subtypes |             |
| Non-WNT/non-SHH    | 12 (63.2)   |
| SHH-activated      | 6 (31.6)    |
| WNT-activated      | 1 (5.3)     |
| MYC Status         |             |
| Amplified          | 2 (10.5)    |
| Not-amplified      | 17 (89.5)   |
| MYCN Status        |             |
| Amplified          | 1 (5.3)     |
| Not-amplified      | 18 (94.7)   |

### Table 2 - MYC and MYCN amplification statuses and outcomes parameters in relation to the histologic subtypes.

| Histological subtypes | MYC amplification | MYCN amplification | Total n (%) |
|-----------------------|-------------------|--------------------|-------------|
|                       | Positive          | Negative           | ND**        | 0 | 2 (10.5) |
|                       | Negative          | 9                   | ND**        | 2 | 6 | 17 (89.5) |
|                       | Positive          | 1                   | ND**        | 0 | 0 | 1 (5.3) |
|                       | Negative          | 8                   | ND**        | 4 | 6 | 18 (94.7) |
| *Metastasis           | 5                 | 0                   | 1            | 1 | 7 (17.9) |
| *Recurrence           | 4                 | 2                   | 2            | 0 | 8 (14) |
| *Mortality            | 2                 | 0                   | 2            | 1 | 5 (8.8) |

*Follow up period is 5 years from the date of diagnosis. **ND - Not done
Medulloblastoma subtypes in Saudi patients… Allassiri et al

cases (5.3 %), which was of the classic subtype. The outcomes of only cases with a follow-up period of 5 years were analyzed. We observed metastasis in 7 patients (5 classic, 1 large cell/anaplastic, and 1 desmoplastic/nodular), tumor recurrence in 8 cases (4 classic, 2 MBLs with extensive nodularity, and 2 large cell/anaplastic) and disease-related death in 5 cases (2 classic, 2 large cell/anaplastic, and 1 desmoplastic/nodular).

The patients’ outcomes in relation to treatment modalities are shown in Table 3. Multimodal therapy (surgery combined with chemotherapy and radiotherapy) was administered to 26 patients (63%). The metastatic rate was higher in cases with subtotal resection (n=3, 21%) compared to those with complete resection (n=4, 10%). The mortality rate was much higher when surgery was the only treatment modality. Tumor recurrence was observed more frequently within the first year after diagnosis. Most of the metastases were present at the time of the initial presentation.

As shown in Figure 1, patients with MYC and MYCN amplifications had significantly shorter survival times compared to the non-amplified cases.

**Discussion.** In our region, the literature is scarce in regards to detailed descriptive studies of MBLs. A very recent study by Alharbi et al found that the WNT-activated MBLs were the least common while group 4 MBLs were the most prevalent based on methylation analysis of 39 cases. Some morphological features can predict the molecular subtype; for example, all nodular/desmoplastic MBLs belong to the SHH-activated subgroup, although not all SHH MBLs are nodular/desmoplastic. Notably, the large-cell/anaplastic subtype is observed in all 4 molecular subgroups and has been shown to indicate a poor prognosis. As a result, patients with large-cell/anaplastic MBLs are treated with more intensive therapy using the high-risk MBL protocol. In the current study, patients with the large-cell/anaplastic subtype of MBL also had poor prognoses.

The MBLs harboring amplifications in the MYC oncogene occur much less frequently (approximately 4% of patients), and are commonly associated with large-cell/anaplastic MBLs, the group 3 molecular subgroup, and poor outcomes. The MYCN amplification, which is also associated with a poor prognosis, preferentially occurs in SHH-driven tumors and is associated with molecular subgroup 4. Analysis of the relevance of MYC oncogene family dysregulation in MBL identified MYC and MYCN amplifications as independent markers of poor progression-free and overall survival. Notably, patients with combined large-cell/anaplastic morphologic subtypes and MYC

**Figure 1** - Kaplan Meier survival curves. a) The 2 MYC amplified (green), one MYCN amplified (blue), and the remaining 15 non-amplified cases (yellow); b) The two MYC amplified cases (blue) versus the remaining 17 non-amplified cases (green). c) The one MYCN amplified case (blue) versus the remaining 18 non-amplified cases (green).
amplification reportedly have very poor prognoses.\(^9\) Consequently, patients whose tumors exhibit MYC and/or MYCN amplification should be considered high-risk. MBLs in children have been studied extensively at the molecular level; however, MBLs in adults are probably genetically distinct, and molecular markers other than MYC and MYCN (such as the copy number status of 10q and 17q) appear to be of more relevant prognostic value.\(^13\) Interestingly, both of our MYC-amplified cases were diagnosed in pediatric patients and had poor prognoses, as is consistent with data from other studies.\(^5,\)\(^12\)

The age limit for pediatric patients at our institute is ≤14 years, and just over half of the patients in our study were treated by pediatric oncologists with a slightly different therapeutic approach than was administered to adult patients. Of note, there would have been a significant overrepresentation of pediatric MBLs had we used the pediatric age limit of 18 years. Treatment protocols for MBLs include surgery that aims to achieve gross total resection (GTR), craniospinal irradiation (CSI), and adjuvant chemotherapy or autologous stem cell transplantation.\(^14\) Most of our patients who underwent GTR also received craniospinal radiation and adjuvant chemotherapy, which is the standard of care for pediatric MBLs.

**Limitations.** Our study had several limitations including the small sample size, out of which only 19 cases were molecularly tested using a limited FISH panel for monosomy 6 and MYC and MYCN amplifications.

In conclusion, our data showed that classic MBL is the most diagnosed histomorphologic subtype and the non-WNT/non-SHH molecular group is the most common molecular subgroup. The percentage of MYC and MYCN amplifications is 10.5% and 5.3% respectively. As expected, the two large cell/anaplastic MBL cases with MYC amplification had the worst outcomes. Further comprehensive molecular testing will allow stratification of the non-WNT/non-SHH subtypes into group 3 and group 4 for survival correlation.

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