The Utility of Phosphohistone H3 in Inter-Observer Variability of Mitotic Count in Meningioma, is There Any Benefit?

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Abstract

Objective: One of the most reliable and decisive histologic parameters with negative prognostic impact is tumor proliferation capacity. Quantification of mitosis in H&E stained slides could be problematic and is limited by poor reproducibility and lack of objectivity. This study was designed to evaluate inter-observer variability in mitotic count using Phosphohistone H3 (PHH3). Methods: Totally, 60 specimens with histologic diagnosis of meningioma were selected including 50 grade I, 7 grade II and 3 grade III tumors. Mitotic figures were counted both in H&E stained sections and slides prepared by immunohistochemistry using Anti-Phosphohistone H3 Anti body by three observers with various level of expertise, independently. Results: Mean mitotic count by PHH3 method was higher than H&E staining for all three observers. Observer 1 and 2 revealed good correlation in mitotic count using H&E method, while observer 3 showed disagreement with both of them. However, all of them had good correlation in mitotic count using PHH3 method (cc=0.956,0.947,0.909). Conclusion: Based on our findings, PHH3 revealed good agreement between pathologists with various level of expertise and has the capability for further contribution in meningioma grading classification and specially could be beneficial for less experienced pathologists.

Keywords: Phosphohistone H3- meningioma- grade- mitosis- inter-observer variability

Introduction

Predicting probable aggressive behavior or recurrence in meningiomas are related to various factors including location, extent of resection, invasion to brain parenchyma and histologic grade.(Elmaci et al., 2018). The three tired grading system by WHO admits risk estimation based on histomorphologic criteria (Kim et al., 2007). Among the negative known prognostic factors in meningiomas, proliferation capacity has been described as one of the most reliable and decisive histopathological parameters (Perry et al., 1997; Kim et al., 2007; Winther et al., 2016). However, quantification of mitosis in Hematoxylin and Eosin (H&E) stained slides could be problematic (Elmaci et al., 2018; Puripat and Loharamataweethong, 2019) and is limited by poor reproducibility and lack of objectivity (Winther et al., 2016). One of the most challenges are mitosis mimickers including chromatin changes seen during apoptosis, pyknotic nuclei, necrotic or crushed cells (Hendzel et al., 1998; Ribalta et al., 2004; Winther et al., 2016; Elmaci et al., 2018; Puripat and Loharamataweethong, 2019). Moreover, heterogeneity of mitotic activity in different areas, variation in cell density and bias in sampling could be other confounding factors (Winther et al., 2016; Elmaci et al., 2018; Puripat and Loharamataweethong, 2019).

Numerous biomarkers and methods have been evaluated for more accurate assessment of the degree of proliferation (Winther et al., 2016). Phosphohistone H3 (PHH3) is a core histone protein, together with other histones forms the major protein constituents of the chromatin in eukaryotic cells. It appears that, PHH3 is negligible during interphase in cell cycle but reaches to maximum condensation during mitosis (Puripat and Loharamataweethong, 2019). Phosphorylation of histone H3 is mitosis specific (Fukushima et al., 2009; Olar, 2015). Immunohistochemically, it stains mitotic figures from early prophase through metaphase, anaphase and telophase (Tapia et al., 2006; Puripat and Loharamataweethong, 2019). Phosphohistone H3 (PHH3) as a mitotic marker is able to distinguish mitotic cells from mitosis mimickers including apoptotic bodies, distorted and crushed cells or from necrotic and karyorrhectic debris (Puripat and Loharamataweethong, 2019). It has been proved reliable in different types of tumors (Baehner and Weidner, 2000;
The alternative biomarkers, Ki67, a non-histone cell cycle protein, has been shown potential utility and been used in clinical practice (Winther et al., 2016). However, ki67, usually overestimates the proliferative activity, because cells in several phases of cell cycle, including M, G1, G2 and S are all stained positive (Puripat and Loharamataweethong, 2019). In addition, tumor infiltrating inflammatory cells also can be positive (Puripat and Loharamataweethong, 2019). So, it appears that PHH3 could be superior to ki67 in some aspects.

Considering the significance of accurate and reproducible mitotic count in grading of meningioma, this study was designed to evaluate whether mitotic count using PHH3 could decrease inter-observer variability between pathologists or not.

Materials and Methods

Totally, 60 specimens with histologic diagnosis of meningioma were selected including 50 grade I, 7 grade II and 3 grade III tumors classified based on WHO classification system (Louis et al., 2016). The slides were reviewed and sections with the highest mitotic activity on H&E, and adequate preserved tumoral tissue were selected. Immunohistochemistry was performed using Rabbit polyclonal anti PHH3 (Biocare Medical) based on manufacturer’s protocol. Tonsil tissue was used as positive control (Figure 1).

Two pathologists (1 and 2) and one senior resident of pathology (3) reviewed the slides separately. They were blind to each other’s scoring.

The mitotic figures were counted in 10 consecutive high power fields in H&E stained slides. Then, IHC slides stained with anti PHH3 antibody were evaluated. Positive Brown stained nuclei with loss of nuclear membrane or presence of chromosome condensation arranged along a plane or separated were considered as positive cells and intact brown stained nuclei or nuclei with smooth membrane and absence of chromosome condensation were excluded (Puripat and Loharamataweethong., 2019) (Figure 1).

All data were gathered and analyzed using SPSS version 22 software, paired sample t-test.

Results

Sixty samples were examined. The mean age among patients in grade I, II and III were 53.3, 63.14 and 69.33 years, respectively.

Mean mitotic count on H&E stained and PHH3 slides are shown in Table 1. The mean mitotic count by PHH3 method was higher for all three observers and the difference was significant for observer 2 and 3.

Observer 1 and 2 revealed good correlation in mitotic count using H&E method, while observer 3 showed significant disagreement with both of them. However, all of them had good correlation in mitotic count using PHH3 method (Table 2).

Observer 1 had one increase in tumor grade (upgrade: 3 mitosis/HPF to 4) and one decrease in grade (downgrade: 4 mitosis/HPF to 3) following incorporating PHH3. Observer 2 faced two upgrades (3 mitosis/HPF to 4 and 5) when using PHH3 in comparison to H&E. However, the results for observer 3 (resident of pathology) was more variable and 4 tumors were up graded and 4 tumors down graded, totally.

Discussion

Meningioma constitutes about one third of the primary

| Observers code | Mean mitotic count in H&E method | Mean mitotic count in PHH3 method | p value | Coefficient Correlation |
|----------------|---------------------------------|---------------------------------|---------|------------------------|
| 1              | 2.40                            | 2.65                            | 0.066   | 0.854                  |
| 2              | 2.26                            | 2.63                            | 0.007   | 0.807                  |
| 3              | 1.76                            | 2.66                            | 0.000   | 0.762                  |

Table 1. Comparison of Mitotic Count Using H&E and PHH3 Methods by Three Observers

| Observers code | H&E | PHH3 |
|----------------|-----|------|
| 1 and 2        | P=0.220; CC=0.923 | P=0.811; CC=0.956 |
| 1 and 3        | P=0.000; CC=0.821 | P=0.811; CC=0.947 |
| 2 and 3        | P=0.000; CC=0.839 | P=0.709; CC=0.909 |

Table 2. Level of Agreement and Correlation of Mitotic Count between Observers in H&E and PHH3 Methods.
intracranial neoplasms. They represent the largest group of central nervous system (CNS) tumors, and have an intimate trend to recur (Puripat and Loharamataweethong, 2019). Tumor grade, proliferative capacity, histologic subtype and extent of resection are various factors influence recurrence rate (Kasuya et al., 2006; Puripat and Loharamataweethong, 2019).

Considering mitosis as one of the most negative prognostic factors (Kim et al., 2007), accurate and precise counting of mitotic figures (MF) might be subjected to inter-observer variability. In-fact, distinguishing MF in H&E stained slides from similar chromatin changes is a subjective task (Kim et al., 2007) and this may result in inconsistencies in tumor grading specially in borderline cases (Puripat and Loharamataweethong, 2019).

Ki67 has been widely investigated in meningioma grading. However, it is not mitosis specific and there is no agreed cut off value (Puripat and Loharamataweethong, 2019). A meta analysis from 53 studies revealed great variation in cut offs recommended to predict recurrence, ranging from 1 to 10% (Prayson, 2005; Olar et al., 2015; Elmaci et al., 2018).

PHH3 immunostaining allows the pathologist to assess morphologic features of mitosis at the same time, increasing the specificity of quantification (Ribalta et al., 2004; Bossard et al., 2006; Casper et al., 2010; Brunner et al., 2012; Elmaci et al., 2018), improvement in reproducibility and/or reduce in inter-laboratory or inter-observer variability (Angi et al., 2011; Draganova-Tacheva et al., 2013; Duregon et al., 2014; Cui et al., 2015; Duregon et al., 2015; Winther et al., 2016; Elmaci et al., 2018). Moreover, because it is mitosis specific (Aune et al., 2011; Braun et al., 2013; Olar, 2015) and stains solely mitotic cells, it appears more comparable with mitotic index compared to ki67 which stains all phases except for G0 (Colman et al., 2006; Elmaci et al., 2018).

Fukushima et al. (2009) reviewed 45 meningioma cases classified based on WHO criteria (37 benign; 7 atypical form and 1 anaplastic). They concluded PHH3 as a reliable tool for discrimination between apoptotic and crushed cells leading to more precise counting of mitotic Figures. In addition, in their study, mitotic count based on PHH3 was significantly greater than mitotic count based on routine H&E method. By PHH3, two tumors with grade I were upgraded in to grade II (Fukushima et al., 2009; Elmaci et al., 2018). In our study, also mean mitotic count was higher in PHH3 method in comparison to H&E staining by all three observers with significant agreement by PHH3 method. Moreover, we observed a robust, easy and reliable method and could potentially decrease interobserver variability specially in less experienced pathologists.

PHH3 staining has the capability for further contribution in meningioma grading classification and specially could be beneficial for less experienced pathologists. Considering mitosis as one of the most negative prognostic factors (Kim et al., 2007), accurate and precise counting of mitotic figures (MF) might be subjected to inter-observer variability. In-fact, distinguishing MF in H&E stained slides from similar chromatin changes is a subjective task (Kim et al., 2007) and this may result in inconsistencies in tumor grading specially in borderline cases (Puripat and Loharamataweethong, 2019).

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Author Contribution Statement

Hana Saffar: Review of slides (Pathologist), preparing the manuscript. Hoda Okhovat: Review of slides (Resident of pathology). Saeed Arabsoleymani: Data analysis and preparing the preliminary draft of the manuscript. Seyed
Mohammad Tavangar: Final preparation and approval of manuscript. Alireza Khosheviavan: Final preparation and approval of manuscript. Ghazal Hajinasrollah: Data analysis. Zahra Hamidi Afra: Data entry and preliminary analysis. Hiva Saffar: Study design, review the slides, final proof of manuscript and corresponding author.

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Conflict of interest

The authors declare that they have no conflict of interest.

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