Assessment of Mitotic Activity in Pituitary Adenomas and Carcinomas

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Abstract

Assessment of mitotic activity represents one of the oldest and most routinely used histopathologic methods of evaluating the biological aggressiveness of human tumors. In the case of pituitary tumors, however, the relevance of this approach as a means of gaging tumor behavior remains ill-defined. In this article, the relationship between the mitotic index and biological aggressiveness of pituitary tumors was evaluated in a series of 54 pituitary adenomas and 6 primary pituitary carcinomas. All tumors were fully classified by immunohistochemistry and electron microscopy; adenomas were further stratified on the basis of their invasion status, the latter being defined as gross, operatively, or radiologically apparent infiltration of dura or bone. Mitotic figures were present in 11 tumors, 10 being either invasive adenomas or pituitary carcinomas. A significant association between the presence of mitotic figures and tumor behavior was noted, as evidenced by progressive increments in the proportion of cases expressing mitotic figures in the categories of noninvasive adenoma, invasive adenoma, and pituitary carcinoma (3.9, 21.4, and 66.7%, respectively; Fisher's exact test, two-tailed, $p < 0.001$). The mitotic index, however, appeared to be a less informative parameter, being extremely low in all cases (mean = 0.016% ± 0.005 [± SEM]). Although the mean mitotic index in pituitary carcinomas (0.09% ± 0.035) was significantly higher than the mean mitotic index of either noninvasive adenomas (0.002% ± 0.002) or invasive adenomas (0.013% ± 0.005), no practical threshold value capable of distinguishing these three groups was evident. Comparison of the mitotic index with Ki-67 derived growth fractions in these tumors revealed a significant but weak linear correlation ($r = 0.41$, $p < 0.01$). These data suggest that when, mitotic figures are present, they do provide some indication of the behavior and invasive potential of pituitary tumors. For routine diagnostic purposes, however, the discriminating power of this parameter is somewhat limited, being superseded by alternative and more informative methods of growth fraction determination such as that provided by the Ki-67 immunolabeling.

Key Words: Pituitary adenomas; pituitary carcinomas; mitotic index; invasiveness; Ki-67; proliferation markers.

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Introduction

Providing prognostically relevant insight into the biological behavior of pituitary tumors remains one of the foremost challenges confronting the pathologist. In tumors arising in other organs, histologic evaluation often permits clinically relevant inferences into tumor aggressiveness. Mitotic activity is a rather generic morphologic parameter, one that, for some tumor types, not only determines the histopathologic grade, but (in some instances) also influences the choice of

therapeutic intervention and reflects a patient's prospects for long-term survival [1–3]. In pituitary tumors, mitotic figures are uncommon. As a result, their practical value in assessing tumor aggressiveness has not been systematically analyzed.

In this article, the mitotic activity in a series of immunohistochemically characterized and ultrastructurally classified pituitary adenomas and carcinomas was evaluated. The biologically relevant endpoint to which mitotic activity was correlated was the tumor's invasion status, defined as gross, radiographically, or intraoperatively apparent infiltration of dura or bone. Gross invasion was chosen because it represents an objective and clinically relevant parameter of tumor aggressiveness, one that contributes to the neurologic and endocrinologic morbidity of pituitary tumors, adversely affecting their successful surgical removal and the patient's prospects for disease-free survival [4–10]. In addition, the mitotic activity was correlated with Ki-67 derived tumor growth fractions, as determined by MIB-1 antibody immunostaining method. The latter has recently been shown to correlate with the invasion status of pituitary tumors [11].

Materials and Methods

Tumor Specimens

Tumor specimens were obtained from 60 patients, including 34 females and 26 males with a mean age of 44.3 yr (range 22–64 yr). All had undergone surgical resection for their pituitary tumor. Included were 54 adenomas and 6 primary pituitary carcinomas. Pituitary adenomas were distinguished on the basis of their gross invasion status as determined by intraoperative findings and/or preoperative magnetic resonance imaging studies. Among the 26 noninvasive adenomas there were 5 microadenomas and 21 macroadenomas. Of the 28 invasive adenomas, all were macroadenomas. Six primary pituitary carcinomas were also included in this analysis; all were invasive lesions exceeding 1 cm in diameter with demonstrated craniospinal and/or systemic metastases. Of these, 3 were prolactin cell carcinomas and 3 were corticotroph carcinomas occurring in the setting of Nelson's syndrome. All tumors had been ultrastructurally classified and were fully characterized, both histologically and immunohistochemically. All major functional and nonfunctional adenoma subtypes were represented (Table 1). In addition to the 60 cases comprising the study set, 10 pituitary glands obtained at autopsy of patients dying of nonendocrine disease served as control tissues.

Counting of Mitotic Figures and Mitotic Index Derivation

Mitotic counts were performed on histologic sections of formalin-fixed, paraffin-embedded tissues. Sections were stained with H&E. Mitotic counts were performed at high power (×400) without knowledge of the secretory type or invasion status of the tumor. Counts were obtained with the aid of a 10 × 10 square grid fitted into the eyepiece of the microscope. Proceeding in a systematic fashion as determined by the grid, a representative sampling of cells was obtained along the full sectional area of the specimen. A minimum of 20 high-power fields were enumerated per specimen; an average of approx 1700 cells were evaluated in each case. The mitotic index was derived as the percentage ratio of the number of mitotic figures to the total number of cells counted.

Table 1. Mitotic Index in Pituitary Tumor Subtypes

Tumor type[a]	*n*	Mean mitotic index, %
Prolactin cell adenoma	4	0.0133
Densely granulated GH cell adenoma	4	0
Sparsely granulated GH cell adenoma	8	0.0071
Mixed GH-PRL cell adenoma	5	0.0076
Mammosomatotroph adenoma	1	0
Acidophil stem cell adenoma	3	0
Corticotroph adenoma	3	0
Silent corticotroph adenoma	3	0
Thyrotroph adenoma	4	0.0221
Gonadotroph adenoma	8	0.0070
Null cell adenoma	5	0.0156
Oncocytoma	6	0.0088
Total adenomas	54	**0.0078**
Prolactin cell carcinoma	3	0.1392
Corticotroph carcinoma (Nelson's syndrome)	3	0.0466
Total carcinomas	6	**0.0929**

[a]GH, growth hormone; GH-PRL, growth hormone-prolactin.

MIB-1 Immunostaining and Derivation of Ki-67 Labeling Index

In all tumors studied, Ki-67-derived tumor growth fractions were determined using the MIB-1 antibody. Data regarding the growth fractions of 40 of the 60 tumors analyzed herein were already available, having been included by the authors in a recent companion publication [11]. New growth fraction data were obtained in the additional 20 cases. Ki-67 immunolabeling was performed on formalin-fixed paraffin-embedded tissues as previously described [12]. Immunostaining was performed using the avidin–biotin–peroxidase complex method of Hsu et al. [13]. Appropriate antigen retrieval methods were employed; details of the method are described in a recent publication by the authors [11]. A monoclonal MIB-1 antibody was used at a 1:50 dilution (AMAC, Westbrook, ME).

Immunostained slides were evaluated at high power (×400) without knowledge of the secretory type, invasion status, or mitotic index of the tumor. Using the same grid apparatus and identical counting strategy as described in the previous section for the mitotic counts, 20 high-power fields were evaluated in each case. For each tumor, a Ki-67 labeling index or growth fraction was determined, expressed as the percentage of Ki-67-labeled nuclei.

Statistical Analysis

Several standard univariate methods of evaluating categorical response data were used in this study. To determine whether an association existed between the presence of mitotic figures and tumor behavior (i.e., noninvasive, invasive, or carcinoma), the Fisher's exact test was employed. In comparing the mean mitotic index in each of these groups, a one-way analysis of variance (ANOVA) was performed, followed by planned pairwise comparisons using the method of Bonferroni. Finally, in order to determine whether a linear relationship existed between the mitotic index and Ki-67-derived growth fraction, the Pearson correlation coefficient was calculated. For each of these analyses, two-tailed probability values of <0.05 were designated as significant. All statistical analyses were performed using *SAS* system software, version 6.10 for the Macintosh (SAS Institute, Cary, NC).

Results

In the anterior lobes of the 10 autopsy pituitary glands studied, no mitotic figures were identified. Mitotic figures were uncommon among pituitary tumors, being present in only 11 of the 60 tumor specimens. When stratified as being either noninvasive adenomas, invasive adenomas, or carcinomas, significant differences were observed in proportion of cases in which mitotic figures were identified: mitotic figures were present in 1 of 26 noninvasive adenomas (3.9%), 6 of 28 invasive

Table 2. Mitotic and Ki-67 Indices in Pituitary Tumors

Tumor group	*n*	Cases with mitotic figures (%)[a]	Mean mitotic index[b]	Mean Ki-67 LI[c]
Noninvasive adenomas	26	1/26 (3.9%)	0.002 ± 0.002%	1.36 ± 0.24%
Invasive adenomas	28	6/28 (21.4%)	0.013 ± 0.005%	4.21 ± 0.66%
Pituitary carcinomas	6	4/6 (66.7%)	0.093 ± 0.035%	13.1 ± 3.78%
Total	**60**	**11/60 (18.3%)**	**0.016 ± 0.005%**	**3.87 ± 0.65%**

[a]A significant association is present between tumor group and the proportion of cases having mitotic figures (Fisher's exact test, two-tailed, $p < 0.001$).

[b]Pairwise comparisons using Bonferroni correction indicate the mean mitotic index in pituitary carcinomas to be significantly higher than either noninvasive or invasive adenomas ($p < 0.025$). No significant difference identified in the mean mitotic indices between noninvasive and invasive adenomas ($p = 1.00$) (one-way ANOVA, F ratio = 19.66, $p < 0.001$).

[c]Pairwise comparisons using Bonferroni correction indicate significant differences in the mean Ki-67 labeling indices between: noninvasive and invasive adenomas ($p < 0.01$), noninvasive adenomas and carcinomas ($p < 0.001$), and invasive adenomas and carcinomas ($p < 0.001$) (one-way ANOVA, F ratio 24.32, $p < 0.0001$).

adenomas (21.4%), and 4 of 6 pituitary carcinomas (66.7%) (Fisher's exact test, two-tailed, $p < 0.001$) (Table 2).

Whereas significant differences were observed in the distribution of mitotic figures among the aforementioned tumor groups, corresponding differences in the mean mitotic index among these groups were far less definitive. The mean mitotic index of all tumors studied was 0.016% ± 0.005 (± SEM, range 0–0.21%) (Table 2). When tumors were stratified, the mean mitotic index among noninvasive adenomas, invasive adenomas, and carcinomas was 0.002 ± 0.002%, 0.013 ± 0.005%, and 0.093 ± 0.035%, respectively. ANOVA confirmed that significant differences were present in the mean mitotic indices between these three groups (one-way ANOVA, F ratio = 19.66, $p < 0.001$). Pairwise comparisons using the Bonferroni method revealed that mean mitotic index in pituitary carcinomas was significantly higher than either noninvasive or invasive adenomas ($p < 0.025$); however, no significant differences in mitotic index were observed between noninvasive and invasive adenomas ($p = 1.00$). Similarly, neither a statistical nor practical difference could be demonstrated between the mean mitotic indices of microadenomas and macroadenomas.

Although the mean mitotic index of hormonally active tumors was higher than that of nonfunctioning tumors (0.02 ± 0.008 vs 0.009 ± 0.005), a statistically significant difference could not be demonstrated (two sample *t* tests for independent samples, $p = 0.183$). There was insufficient statistical power to demonstrate significant differences in the mean mitotic index between the major tumor immunotypes (Table 1).

When comparing the mitotic index with Ki-67-derived growth fractions, several relationships were apparent (Table 2). Incremental changes in the mean mitotic index among the noninvasive, invasive, and carcinoma groups were associated with corresponding differences in the mean Ki-67 labeling index. For noninvasive adenomas, invasive adenomas, and pituitary carcinomas, the mean Ki-67 derived growth fractions were 1.36 ± 0.24%, 4.21 ± 0.66%, and 13.1 ± 3.78%, respectively. Although a significant linear correlation between the mitotic index and the Ki-67-derived growth fraction was observed, the association was not strong ($r = 0.41$, $p < 0.01$).

Highlighting the limitations of this association was the fact that, in the 49 cases in which the mitotic index was 0, the Ki-67-derived growth fractions ranged from 0–16.44%. When considering only the 11 tumors in which mitotic figures could be identified, the linear correlation between these two indices was neither strong nor significant ($r = 0.258$, $p = 0.44$).

Discussion

Of the morphologic criteria classically used to assess tumor aggressiveness, evaluation of mitotic activity is regarded as among the most important. In this study, we have demonstrated that mitotic figures are not a feature of the normal pituitary gland; nor for that matter are they present in substantial numbers in most pituitary tumors. Of practical importance, however, was the observation that when tumors of increasing biological aggressiveness were compared, a corresponding and statistically significant increase in the proportion of tumors exhibiting mitotic figures was observed; mitotic figures were demonstrable in only 3% of noninvasive adenomas, but were present in 21% of invasive adenomas, and in 67% of pituitary carcinomas.

Whereas these data demonstrate a significant association between the presence of mitotic figures and the biological aggressiveness of pituitary tumors, further quantification and the calculation of a mitotic index provided little additional insight into tumor behavior. Although the mean mitotic index of pituitary carcinomas was significantly higher than that of either noninvasive or invasive adenomas, the practical importance of this difference is limited in view of the rarity of pituitary carcinomas. The distinction of greater practical importance, that of differentiating adenomas with and without invasive tendencies, could not be made on the basis of the mitotic index. The limitations of the mitotic index as a biologically informative parameter in pituitary tumors primarily relate to a problem of magnitude. In all pituitary tumors wherein mitotic figures were found (be they noninvasive adenomas, invasive adenomas, or pituitary carcinomas), the mitotic index was uniformly low (mean = 0.089%) and the range of individual values was extremely narrow (0.038–0.213%). Most importantly, clinically relevant differences in tumor behavior were associated with seemingly negligible changes in the mitotic index. Accordingly, invasive and noninvasive adenomas could not be separated. These factors may also account for the poor correlation observed between the mitotic index and the Ki-67-derived growth fraction as determined by the MIB-1 antibody. The latter has recently been shown to be a specific and moderately sensitive predictor of invasiveness in pituitary tumors [11]. Because the Ki-67 antigen is expressed during G1, S, G2, and M phases of the cell cycle, it more accurately represents the true population of cycling cells. Mitotic figures, given their relatively brief appearance during the cell cycle, represent only a small fraction of this population. Therefore, in contrast to the mitotic index, growth fractions based on Ki-67 are not only larger, but their range of values is wider, incorporating the necessary variability to reflect and discern biologically relevant differences in tumor behavior. These findings further corroborate the observations of Kramer et al. [14], wherein mitotic counts failed to correlate with pituitary tumor growth fractions determined by either Ki-67 or proliferating cell nuclear antigen immunostaining.

In summary, assessment of mitotic activity plays a limited role in the evaluation of pituitary tumors. Because mitotic figures are absent in the majority of these

lesions, the finding of any mitotic figures in a pituitary tumor specimen might alert the pathologist to a potentially aggressive tumor with invasive tendencies. The absence of mitotic figures, however, provides no assurance that the tumor is biologically indolent. It should also be emphasized that quantification of mitotic activity in the form of a mitotic index is a laborious undertaking that, in the context of this analysis, failed to provide additional, practical insight into the invasive potential of pituitary adenomas. Although our study demonstrated that the mean mitotic index of pituitary carcinomas was significantly higher than that of either invasive or noninvasive adenomas, no clear threshold value capable of separating adenomas from carcinomas was evident. Whereas intuition and experience with other human tumors would suggest that a higher mitotic index is an indicator of greater biological aggressiveness, this could not be validated for benign pituitary adenomas in this study. Failure to do so may have been owing to the overall rarity of mitotic figures in pituitary adenomas. As only 7 of 54 pituitary adenomas exhibited one or more mitotic figures, this sample size was perhaps too small to demonstrate such a trend.

In general, assessment of mitotic activity is of limited utility in the evaluation of pituitary tumors. Since mitotic figures are present in only a minority of pituitary tumors, and then only in small numbers, they cannot be regarded as robust or routinely relevant predictors of tumor behavior. This limitation further emphasizes the importance of alternative methods of gaging tumor proliferation. Growth fraction estimates, such as are provided by markers like Ki-67, provide a more informative means of assessing tumor aggressiveness, thereby obviating reliance on mitotic indices alone.

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