Cytohistological correlation in pituitary tumor and immunological assessment with the help of Ki-67

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ABSTRACT
Background: Tumors of the pituitary gland and sellar region represent approximately 10–15% of all brain tumors. Pituitary adenoma (PA), the most common pathology of the pituitary gland, can be effectively subclassified only with the help of immunohistochemistry (IHC). This is important and needed for individual patient management.

Aims and Objectives: The objective of the study was to analyze the importance of intraoperative imprint smear cytology and correlating with final histopathological diagnosis. Furthermore, to classify the different types of PA with the help of IHC, prolactin (PRL), adrenocorticotropic hormone (ACTH), and growth hormone (GH) and to predict the benign, atypical, or malignant nature of the tumor with the help of prognostic marker Ki-67.

Materials and Methods: A prospective study was done in 34 cases. The patients whose pituitary gland samples are referred from the endocrine and the neurosurgery department to the pathology department for histopathological examinations were selected. We have studied the clinical features, radiology and touch imprint cytology, histopathology, and IHC with the help of PRL, ACTH, GH, and Ki-67 of PA over 2 years.

Results: In our study, we had 32 cases of PA of 34 cases over a span of 2 years. We have seen that there is a correlation between cytological and histological diagnosis of the subtypes of PA in 62% cases, and the Kappa statistics show a moderate extent of agreement (Kappa - 0.320, 95% confidence interval = 0.031–0.609). Ki-67 when compared to the radiological grading showed a high degree of comparability (Chi-square test: \( P < 0.001 \)). All cases with invasion had a higher Ki-67. On using the Fisher’s exact test, we found that the Ki-67 expression with GH-producing adenomas and ACTH-producing adenomas was comparable (\( P = 1.000 \)) while in PRL-producing adenomas too this was not significant (\( P = 0.269 \)).

Conclusion: PA can be effectively classified with the help of IHC. Intraoperative cytology is important in diagnosing PA, but histopathology remains the gold standard in diagnosing and differentiating PA from other pathologies of the pituitary gland. The radiological grading together with immunological classification and the prognostic markers of Ki-67 is important in deciding the benign or atypical nature of the adenomas thus helping in better patient management.

KEY WORDS: Immunohistochemistry, imprint cytology, Ki-67, pituitary adenoma

Introduction
In fact, pituitary adenomas (PAs) represent the third most common primary intracranial tumor outnumbered only by gliomas and meningiomas. The prevalence of clinically diagnosed PAs has recently been recognized to be 3.5–5 times more frequent than formerly thought with a study from Belgium showing a prevalence of approximately 1/1000 individual.

In 2004, the WHO published a classification system for pituitary tumors based on immunohistochemistry (IHC) distinguishing...
them according to the presence or absence of secretory products along with various other ultrastructural features."\(^{[3]}\) Apart from benign typical adenomas and pituitary carcinomas, this classification also identified atypical adenomas as tumors with “atypical” morphological features suggestive of an “aggressive behavior” substantiated further by the presence of invasive growth, high mitotic index, Ki-67 labeling index (LI) >3% as well as extensive nuclear staining for p53.\(^{[4]}\)

### Materials and Methods

This is a prospective and observational study conducted at the Department of Endocrinology and Neurosurgery and Department of Pathology in our hospital over a span of 2 years (September 2012–August 2014) with a total of 34 cases. Patients presenting with the signs and symptoms suggestive of any of the PAs (either functional or nonfunctional) and selected for operation were selected for our study.

Radiological parameters are the size of tumor and extent of bone invasion (Hardy grading).\(^{[5]}\) The laboratory parameters assessed were relevant hormone estimations, e.g., prolactin (PRL) and growth hormone (GH). Imprint smears were made intraoperatively from fresh samples and stained with May-Grunwald-Giemsa stain for air-dried smears and Papanicolaou stain for alcohol-fixed smears. Cytological findings were compared with subsequent histopathology report, taking histopathology as the gold standard.

IHC is done with the help of Ki-67. Ki-67 LI <3% is taken to be noninvasive adenoma.\(^{[5]}\) The PRL, GH, adrenocorticotropic hormone (ACTH), and IHC were measured by cytoplasmic positivity of these hormones in the tissue sample, where cytoplasmic staining in ≥10% of cell population was taken to be positive for that hormone.

### Results

Most cases 20/32 (65%) were in the 40–70 years age group, seven cases were in the 30–39 years age group, and five cases were in the 20–29 years age group. We had 32 cases of PA with one case of Rathke’s cleft cyst and one epidermoid cyst. One patient in our study (1/32) was found to have pituitary apoplexy. Because of totally hemorrhagic and necrosed sample, no further histological or immunological subtyping could be conducted on this.

Intraoperative touch preparations show abundant exfoliation of a monotonous population of cells. In our study, in 100% cases, the touch imprint was able to accurately diagnose a PA, differentiating it from other pathologies, as well as normal pituitary gland. In 62% cases, the cytology could correctly predict the histological type. Cytological findings in our study show acidophilic, basophilic, and chromophobic variants [Figure 1].

The application of Kappa statistics shows moderate extent of agreement between cytology and histology with regard to subtyping of the PA (Kappa = 0.320, standard error = 0.147, and 95% confidence interval = 0.031–0.609) [Table 1]. The histological pattern seen is the cells being diffusely arranged in sheets and clusters. In some cases, a papillary arrangement is seen [Figures 2 and 3]. However, the patterns are not prognostically important.

Radiology is useful in assessing the tumor size and tumor invasion. Microadenomas remain confined to the sellar space are designated Grade 0 or Grade I if there is slight sellar enlargement. Macroadenomas are graded on a II–IV scale, with Grade II for tumors with diffuse sellar enlargement but no bone erosion, Grade III for focal bone erosion, and Grade IV for extensive bone erosion including skull base extrasellar structure.\(^{[6]}\)

Invasiveness is defined as extension into the sellar floor bone, cavernous sinus, and/or diaphragma sella, as assessed on preoperative neuroimaging studies.\(^{[7]}\) IHC of the 31 PA was done with Ki-67, PRL, GH, and ACTH. The IHC was conducted on 31 cases (excluding one case of pituitary apoplexy), and GH-producing adenomas (GH-omas) were the most common subtype (36%) in our study. PRL-producing adenomas (PRL-omas) were 19%, mixed GH + PRL adenomas were 16%, and ACTH-producing adenomas (ACTH-omas) were 10%. These hormones showed a cytoplasmic positivity. There were 19% cases which did not stain with any of these stains. However, as we have not considered the other stains such as thyroid-stimulating hormone, lutetinizing hormone, or follicle-stimulating hormone, we have not labeled them as null cell adenomas. The reason we have included GH, PRL, and ACTH only in our study is because they are the most common hormones to produce a symptomatic adenoma due to hormone excess. On IHC, Ki-67 LI is <3% in all cases of PA [Figure 4] except four cases (13%) were above 3%, the cutoff for Ki-67. Ki-67 when compared to the radiological grading showed a high degree of comparability (Chi-square test: P < 0.001). All cases with invasion had a higher Ki-67.

### Statistical analysis

Software used in this study is MedCalc version 11.6 (MedCalc Software 2011, Mariakerke, Belgium).

### Discussion

In our study over a span of 2 years, we found 34 cases of pituitary tumors of which 32 turned out to be PAs. In a 10-year study conducted in Germany, Saeger et al. have published 4122 cases.\(^{[7]}\)
As highlighted by Kleinschmidt-DeMasters, the most common age group of PAs is 30–70 years and that corroborates with our findings. We have found that most patients presented with visual difficulties and mass effects (65%). A study by Rishi et al., the most common presenting symptoms were also visual symptoms and headache.

During the estimation of serum biochemical markers, we have found that PRL is the most commonly raised hormone in the serum 31.3% (10/32). However, 56.3% cases (18/32 patients) are found to have no raised serum hormones (nonfunctioning PA). When the serum hormone levels were compared with the final IHC subtype of the PA, there was corroboration in only 32% (10/31) cases in our study. This showed that serum hormone levels are imprecise indicators of the immunological type of PA.

In a study by Ortiz-Plata et al., PRL-omas are 86%. In the study by Dumbrava et al., also, discordances between serum biochemistry and IHC have been highlighted. In their study on 34% cases, a perfect correlation has been seen between the two modalities.

Radiology is useful in assessing the tumor size and tumor invasion. We have found that Grade II tumors to be the most common (67.74%) using Hardy’s classification, and Grade III tumors having invasion were seen in 9.4% cases, the rest being Grade I tumors (22.6%). A study by Ortiz-Plata et al. has revealed that 18% cases belong to Grade I and II with the majority being Grade IV.

Ki-67 and p53 are prognostic markers of PA. Ki-67 (MIB-1 LI) was calculated using 3% as the cutoff point between benign and atypical/invasive adenomas. In our study, only 13% (four patients) were above 3% and 87% were benign adenomas. Mahta et al. have found that in only five cases of 85 patients (5.9%), MIB-1 LI was more than 5% of which two cases were invasive. When comparing Ki-67 expression with radiological subtypes, a high degree of significance was found (Chi-square test: $P < 0.001$) in our study. All of our Grade I tumors had a Ki-67 level <3%, Grade II mainly had Ki-67 levels <3% while all the Grade III PAs with invasion showed Ki-67 levels above 3%. Thus, invasion is a good determinant of Ki-67 values.

According to the WHO, pituitary tumors are rarely seen in the pediatric population. We have also not encountered any patient in the pediatric age group in the course of our study. As highlighted by Kleinschmidt-DeMasters, the most common age group of PAs is 30–70 years and that corroborates with our findings. We have found that most patients presented with visual difficulties and mass effects (65%). A study by Rishi et al., the most common presenting symptoms were also visual symptoms and headache.

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findings of Ki-67 >3%, 10% are atypical adenomas and the rest are benign adenomas according to the WHO in our study. We have not received a single case of pituitary carcinoma. Saeger et al. have reported 2.7% atypical adenomas and 0.12% pituitary carcinomas. [14] Hence, atypical adenomas and pituitary carcinomas are extremely rare. Thapar et al. [15] reported that a 3% Ki-67 LI cutoff value is associated with 72.7% sensitivity and 97.3% specificity and a positive and negative predictive value of 96% and 80%, respectively, in distinguishing noninvasive from invasive PAs. As study spanning is short, an increased number of cases will probably give us a comparable data with the other studies, pertaining to invasive adenomas.

In our study, Fisher’s exact test was carried out in GH-omas and ACTH-omas, the Ki-67 expression with GH-omas & ACTH-omas was comparable (P value 1.000), and in PRL-omas, this test was not significant (P = 0.269). Thus, Ki-67 values do not particularly relate with any particular type of adenoma. We have found that PRL-omas and mixed PRL + GH adenomas have a slightly lower Ki-67 while ACTH-omas have a slightly higher range of Ki-67. In another study by Pizarro et al., Ki-67 was positive in 139/159 patients (87%) and the MIB-1 index ranged from 0.16% to 15.48% being higher in ACTH-omas. [15]

The therapy of aggressive PAs is nowadays challenging. When surgery and medical therapy fail, radiotherapy becomes the treatment of choice. Recent case reports using temozolomide have provided early encouraging results. [16] [17]

**Conclusion**

Thus, PA, the commonest pathology of the pituitary gland can be effectively subclassified only with the help of IHC. The radiological grading, together with the prognostic markers of Ki-67 & p53 are important markers which help in deciding the benign or atypical nature of the adenomas, thus helping in better patient management & predicting recurrences.

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**Conflicts of interest**

There are no conflicts of interest.

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