

## The Importance of Immunohistochemical Analysis in Silent Pituitary Adenoma

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### Abstract

Pituitary adenoma contributes to 15% of all intracranial neoplasm. It is usually following benign course and some of them are silent (asymptomatic clinically, but hormone-secreting). Silent adenoma usually found incidentally or when the patients show mass effect (neurological deficits). Many of histologically aggressive silent adenoma subtypes are associated with invasiveness, recurrence and progression to clinically functioning adenomas. Aggressive silent adenoma radiologically tends to invade in downward direction, invading bone, sinus cavernosus, parasellar region. The nature of aggressive silent adenoma subtypes is differing in nature compared to benign nature of pituitary adenoma and should be confirmed immunohistochemically to determine the prognosis and anticipate the risk of recurrence or progression. The case illustration show a real case of 46 years old female progressive headache and visual disturbance diagnosed with non-functional pituitary macroadenoma but positive for more than one immunochemistry biomarker (plurihormonal aggressive silent adenoma).

**Keyword :** Silent adenoma, pituitary adenoma, immunohistochemical analysis

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### Introduction

Pituitary adenomas affect 15-20 people per million population per year and contribute 15% of all intracranial neoplasm.<sup>1</sup> Since pituitary adenomas usually following benign course and silent variant doesn't cause any clinical syndrome of hormone affected, usually silent adenoma found incidentally during head imaging or when adenoma is large enough to cause neurological symptoms, such as headache (66%), bitemporal hemianopia (87.2%), cranial nerve palsy (8%), increased intracranial pressure, and pituitary apoplexy.<sup>1-4</sup>

Silent or clinically nonfunctioning pituitary adenoma is a variant of pituitary adenoma that doesn't cause clinical syndrome of the hormone affected.<sup>2</sup> It can be either totally silent (doesn't cause elevation of serum hormone concentration) or clinically silent (asymptomatic despite supranormal hormonal serum).<sup>2</sup> However, mild hyperprolactinemia (<200ng/mL) is not conclusive of prolactinoma, since mild hyperprolactinemia usually caused by sellar mass that compress stalk and interrupt the blood flow that regulate inhibition of prolactin.<sup>5</sup>

The existence of silent adenomas coming into light after immunohistochemical (IHC) analysis were introduced in 1979-1980 and keep developing.<sup>6</sup> The prevalence of silent adenoma keep increasing because many patients that previously diagnosed as non-functional adenomas were actually immunopositivity in IHC analysis with at least one gonadotropin subunit.<sup>1</sup> It is deduced that silent adenoma morphologically tends to have smaller secretory granules compared to secreting adenomas.<sup>1</sup> Small silent adenomas needs to be observed clinically and by MRI since 20% of silent adenoma will increase in size and cause neurological deficits.<sup>2</sup> Patients with neurological deficits will benefit from transphenoidal surgery of adenoma.<sup>2</sup>

Annually, pituitary adenoma affect 80-100 people per 100,000 population and accounts for 15-30% of pituitary adenoma.<sup>6</sup> In UK, silent adenomas are found in 21,7 people per 100,000 population.<sup>2</sup> Meanwhile, in Japan silent adenomas are found in 3-27% autopsy studies.<sup>1</sup> Silent gonadotroph adenomas are the most commonly found silent adenomas (43-64% silent adenoma, 10% all pituitary adenoma) especially in men, followed by null-cell adenoma (44.4%) in the second place.<sup>1,2</sup> Even though, silent gonadotroph adenoma is the most common silent adenoma compared to silent corticotroph adenoma, histologically 2 out of 3 silent adenoma are morphological similar to corticotroph adenomas in Cushing's disease.<sup>7</sup>

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Silent corticotroph adenoma contribute to 2.9-5.7% of all pituitary adenoma, 3-19% nonfunctioning adenoma, and 20% of all corticotroph adenoma.<sup>1, 8</sup> Silent corticotroph adenomas are caused by mutation in TPIT mRNA and overall more aggressive and have high recurrence compared to other silent adenoma (up to 60%)<sup>1, 2, 7, 8</sup> Plurihormonal adenomas are rarely found and only comprises 1.8% of all silent adenoma cases.<sup>2</sup> Plurihormonal adenomas are classified into 3 types (type 1 morphologically resemble somatotroph adenoma, type 2 morphologically resemble gonadotroph adenoma, and type 3 silent adenoma/silent adenoma subtype 3).<sup>1, 2</sup>

**Case Illustration**

The patient, non-smoker and non-alcoholic female 46 years old came visiting neurosurgery department because of progressively worsen visual defect in both eye since 6 month ago. The patient complained diffuse, dull headache progressively getting worse since 3 years ago. Patient said that her visual defect and dull headache impede patient daily activities. Patient used hormonal contraception by

intramuscular injection 18 years ago, and stopped 9 years ago because of ovarium cyst, which is not operated. Patient started her menstrual cycle at 10 years old and ended at 44 years. Her general condition is unremarkable. The focused neurological and eye examination shown decrease in visual acuity and bitemporal hemianopsia, with right eye was slightly better compared to left eye. The routine laboratory and hormonal panel results were normal except slightly increased prolactin. Head MRI with contrasts shown a large mass absorbing contrast with size  $\pm 3.9 \times 3.7 \times 4.9$  cm in sellar region compressing optic chiasm and invading right parasellar region and pre-pontine cistern suggestive of pituitary macroadenoma (Figure 1). Transphenoidal tumor resection was done with no complication.

The pathologic examination with Hematoxylin Eosin stain show uniformly arrange acidophilic cell with round nuclei and chromophobic cytoplasm, diffuse hypercellularity with alveolar like structure. IHC analysis results were immunopositively for growth hormone (GH), adrenocorticotrophic hormone (ACTH), prolactin (PRL), chromogananin, and Ki-67 (Figure 2).

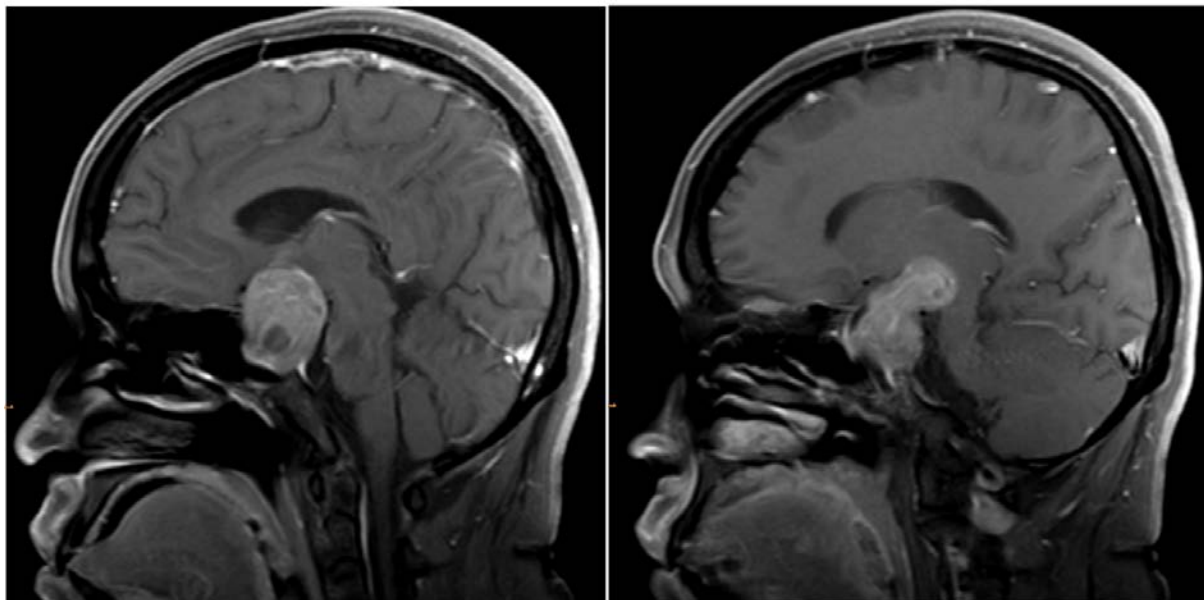
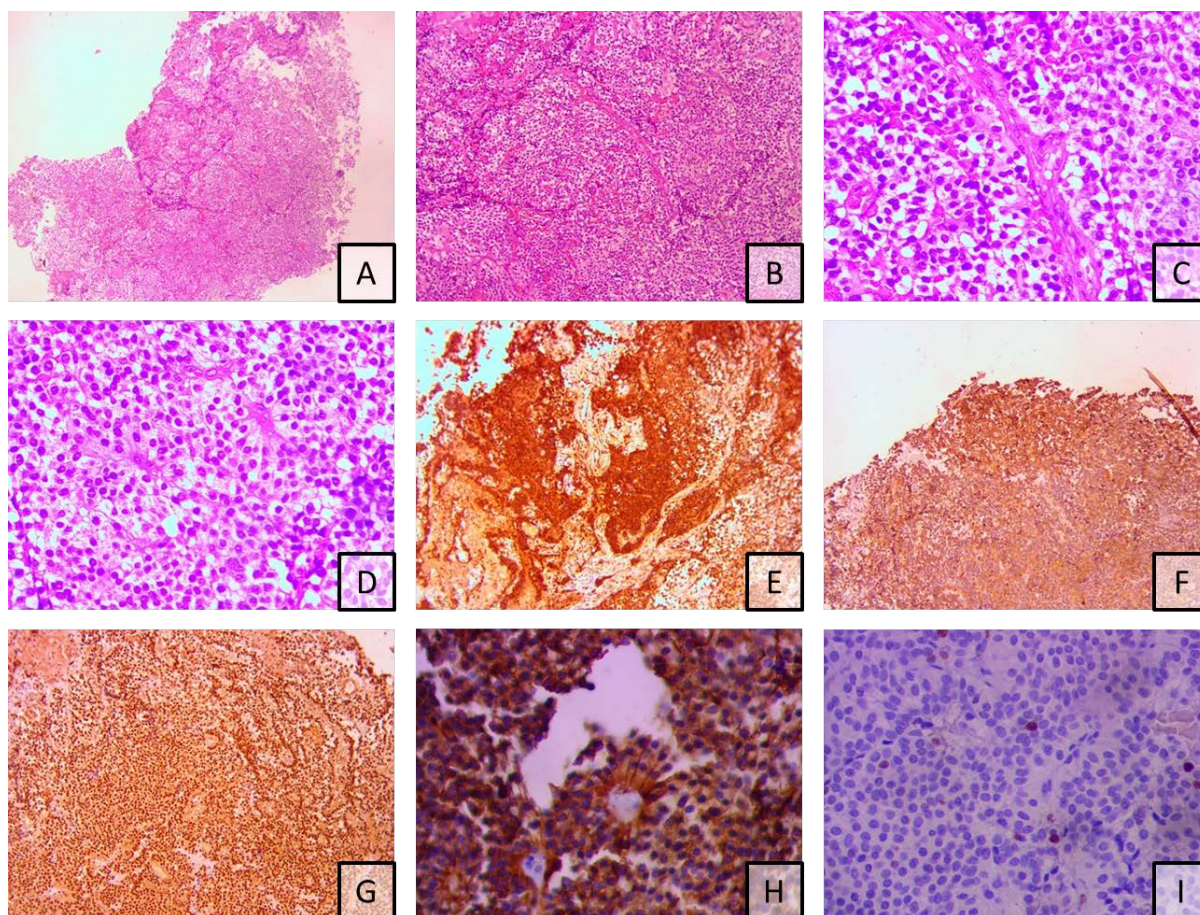


Figure 1. Saggital view of MRI head with Gadolinium contrast showing large mass absorbing contrast with size  $\pm 3.9 \times 3.7 \times 4.9$  cm in sellar region compressing optic chiasm and invading right parasellar region and pre-pontine cistern



**Figure 2.** (A) HE stain 4x, (B) HE stain 10x, (C) & (D) HE stain 40x, (E) IHC PRL (+), (F) IHC GH (+), (G) IHC ACTH (+), (H) IHC Chromogranin (+), (I) IHC Ki-67 (+) 10%  
**Abbreviation :** HE=Hematoxylin-Eosin, IHC=Immunohistochemical analysis, PRL=Prolactin, GH=Growth Hormone, ACTH= Adrenocorticotrophic hormone

## Discussion

It is common to categorized silent adenoma based on adenoma morphological resemblance to corticotroph adenoma into 3 common subtype: Silent 'corticotroph' adenoma subtype I, silent 'corticotroph' adenoma subtype II, and silent adenoma subtype 3.<sup>1, 2, 7</sup> Silent adenomas subtype 1 are morphologically and immunohistochemically indistinguishable with corticotroph adenoma.<sup>7</sup> This subtype of adenoma densely granulated, less common (1.1% all pituitary adenoma), and pose more risk of hemorrhage and infarction.<sup>2, 7</sup> Silent adenomas subtype 2 are chromophobic, sparsely granulated compared to subtype 1, more common (4,4%), higher recurrence rate, greater invasion, and tendency to develop pituitary apoplexy.<sup>2, 3, 7</sup> A cohort study of 25 silent corticotroph adenomas (SCAs) showed that these tumors have a high recurrence rate of 63% compared to 38% in non-functioning adenomas. SCAs also recurred five years earlier than non-functioning adenoma, and de novo recurrences are seen more commonly in

patients with SCA.<sup>9</sup> However another study showed that despite SCAs aggressiveness, it is less likely to recur after tumor resection.<sup>10</sup> Incidence of SCAs recurrence into functional corticotroph adenomas (Cushing's disease) are rare, but found in some case reports.<sup>11-13</sup>

Silent adenoma subtype 3 are rare plurihormonal tumor that accounts for 0.9% of all adenomas, most frequent non-functioning adenoma subtype in patients younger than 25 years old, with mean age 44.3 years (ranging from 13-75 years old).<sup>1-3</sup> It tends to clinically aggressive, not always silent, and actively secreting hormone, but type of hormone secreted needs to be confirmed.<sup>2, 7</sup> Previous study documented that silent adenoma subtype 3 have 31% recurrence in 1-14 years at 29 patients after initial surgery.<sup>14</sup> Newer studies supported by Mayo Clinic show 59% recurrence rate, which 37% of them need re-operation of tumor.<sup>15</sup> Until now, histogenesis of this subtype still debated, but current theory state that it arises from poorly differentiated monomorphous plurihormonal Pit-1 lineage.<sup>3, 7</sup>

All of the patient documented present with macroadenoma (mean diameter 2.8 cm) and prominent mass effects that could invade sinus cavernosus and clivus.<sup>1, 3</sup> Women with this subtype present at younger age with hyperprolactinemia and irregular cycle, meanwhile men will show acromegaly or hyperprolactinemia.<sup>1</sup> Pathological examination with HE stain will show diffuse solid growth in spindle or epitheloid shape, cells with chromophobic or eosinophilic cytoplasm, pleiomorphic nuclei, intranuclear pseudoinclusion, and immunopositive for at least one hormone (GH, prolactin, TSH, or all immunopositive).<sup>1, 2, 7</sup> Besides IHC analysis, electron microscope are mandatory for conclusive diagnosis for silent adenoma subtype 3.<sup>7</sup>

Null cell or non-secreting adenomas don't stain with any pituitary hormone immunonegative or immunopositive to FSH, LH, or  $\alpha$ -subunit of gonadotropin in IHC analysis.<sup>2, 7, 16</sup> Null cell adenoma commonly found in adults and could be classified into two types, oncocyctic types (oncocytomas) which is more commonly found compared to nononcocyctic type.<sup>1, 7</sup> Histologically, this tumors have varying differentiation of cell from chromophobic to eosinophilic, granular (oncocyctic) appearance, in diffuse pattern or pseudorosette formation.<sup>7</sup> Sometimes in more differentiated cell, it is difficult to differentiate oncocytomas and gonadotroph adenoma since oncocytomas arise from the same gonadotroph origin.<sup>7, 16</sup> Electron microscope of null cell adenoma will show a sparse and small secretory granules which released small amount of  $\beta$ -FSH or  $\beta$ -LH or  $\alpha$ -subunit of gonadotroph hormone which can only be detected by avidin-biotin-peroxidase (ABC) method.<sup>16</sup> As the ABC method increasing, the misdiagnosis of silent gonadotroph adenoma as null cell adenoma can be avoided.<sup>16</sup>

Chromogranin A (CgA) is a glycoprotein that presents in the granules of neuroendocrine cells, and expressed in neuroendocrine tumors such as pheochromocytomas, gastrinomas, thyroid medullary cancers, and carcinoid tumors. CgA was found positive in all gonadotropin adenomas, all null cell adenomas,

half of corticotropin adenomas, but rarely observed in GH/PRL-secreting adenomas.<sup>17</sup>

Ki-67 labeling index (LI) are most commonly used biomarker to determine the aggressive-invasive type adenomas and as prognosis marker for recurrence or progression after surgery.<sup>18, 19</sup> Previous study established Ki-67 index >3% as threshold of invasive adenomas with 97% specificity and 73% sensitivity. However, this threshold is not predictive of cavernous sinus invasion compared with other type invasion.<sup>19</sup> The mean value of Ki-67 in patient with recurrent adenomas don't give significant difference and is still inconclusive.<sup>18</sup> Since use of Ki-67 as invasiveness, recurrence, and progression predictor is still controversial, newer study suggested that histological finding is a better predictor of invasiveness of adenoma.<sup>18, 19</sup> Histological subtypes that was associated with aggressive behavior are sparsely granulated somatotroph adenomas, densely granulated lactotroph adenomas, acidophil stem cell adenomas, thyrotroph adenomas, sparsely granulated corticotroph adenomas, silent, subtype 3 adenomas, and null cell adenomas.<sup>5, 20</sup> Commonly this histological subtypes found as macroadenomas that growth and more likely to invade downward structure with bone invasion and parasellar extension, compared to suprasellar invasion.<sup>5, 19</sup>

This illustrative case need to be closely followed with contrast MRI, when there is an evidence of recurrence or aggressive behavior, then radiotherapy is necessary.

### Conclusion

Histopathological examination and immunochemical analysis is necessary to be done for every case of pituitary adenoma, especially silent adenoma to anticipate aggressive-invasive nature of silent adenoma and high risk of recurrence and progression.

### Conflict of Interest

There is no conflict of interest.

### Reference

1. Cooper O, Melmed S. Subclinical hyperfunctioning pituitary adenomas: the silent tumors. *Best practice & research Clinical endocrinology & metabolism.* 2012;26(4):447-60.
2. Mayson SE, Snyder PJ. Silent (clinically nonfunctioning) pituitary adenomas. *Journal of neuro-oncology.* 2014;117(3):429-36.

3. Mete O, Gomez-Hernandez K, Kucharczyk W, Ridout R, Zadeh G, Gentili F, et al. Silent subtype 3 pituitary adenomas are not always silent and represent poorly differentiated monomorphous plurihormonal Pit-1 lineage adenomas. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc.* 2016;29(2):131-42.
4. Vargas G, Gonzalez B, Ramirez C, Ferreira A, Espinosa E, Mendoza V, et al. Clinical characteristics and treatment outcome of 485 patients with nonfunctioning pituitary macroadenomas. *International journal of endocrinology.* 2015;2015:756069.
5. Mete O, Asa SL. Clinicopathological correlations in pituitary adenomas. *Brain pathology.* 2012;22(4):443-53.
6. Chanson P, Raverot G, Castinetti F, Cortet-Rudelli C, Galland F, Salenave S, et al. Management of clinically non-functioning pituitary adenoma. *Annales d'endocrinologie.* 2015;76(3):239-47.
7. Salehi F, Vidal S, Horvath E, Kovacs K, Scheithauer BW. Tumor in the Adenohypophysis. In: Lloyd RV, editor. *Endocrine Pathology:: Differential Diagnosis and Molecular Advances*: Springer New York; 2010. p. 73-89.
8. Cooper O. Silent corticotroph adenomas. *Pituitary.* 2015;18(2):225-31.
9. Cooper O, Ben-Shlomo A, Bonert V, Bannykh S, Mirocha J, Melmed S. Silent corticogonadotroph adenomas: clinical and cellular characteristics and long-term outcomes. *Hormones & cancer.* 2010;1(2):80-92.
10. Bradley KJ, Wass JA, Turner HE. Non-functioning pituitary adenomas with positive immunoreactivity for ACTH behave more aggressively than ACTH immunonegative tumours but do not recur more frequently. *Clinical endocrinology.* 2003;58(1):59-64.
11. Baldeweg SE, Pollock JR, Powell M, Ahlquist J. A spectrum of behaviour in silent corticotroph pituitary adenomas. *British journal of neurosurgery.* 2005;19(1):38-42.
12. Yokoyama S, Kawahara Y, Sano T, Nakayama M, Kitajima S-i, Kuratsu J-i. A case of non-functioning pituitary adenoma with Cushing's syndrome upon recurrence. *Neuropathology.* 2001;21(4):288-93.
13. Psaras T, Honegger J, Buslei R, Saeger W, Klein D, Capper D, et al. Atypical type II silent corticotrophic adenoma developing into Cushing's disease upon second recurrence. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association.* 2007;115(9):610-5.
14. Horvath E, Kovacs K, Smyth HS, Cusimano M, Singer W. Silent adenoma subtype 3 of the pituitary--immunohistochemical and ultrastructural classification: a review of 29 cases. *Ultrastructural pathology.* 2005;29(6):511-24.
15. Erickson D, Scheithauer B, Atkinson J, Horvath E, Kovacs K, Lloyd RV, et al. Silent subtype 3 pituitary adenoma: a clinicopathologic analysis of the Mayo Clinic experience. *Clinical endocrinology.* 2009;71(1):92-9.
16. Kontogeorgos G, Thodou E. The gonadotroph origin of null cell adenomas. *Hormones (Athens, Greece).* 2016;15(2):243-7.
17. Kunert-Radek MPAAGAMRAJ. Chromogranin A in pituitary adenomas: immunohistochemical detection and plasma concentrations 2004. 245-7 p.
18. Dubois S, Guyetant S, Menei P, Rodien P, Illouz F, Vielle B, et al. Relevance of Ki-67 and prognostic factors for recurrence/progression of gonadotropic adenomas after first surgery. *European journal of endocrinology.* 2007;157(2):141-7.
19. Mete O, Ezzat S, Asa SL. Biomarkers of aggressive pituitary adenomas. *Journal of molecular endocrinology.* 2012;49(2):R69-78.
20. Asa SL, Ezzat S. The pathogenesis of pituitary tumors. *Annual review of pathology.* 2009;4:97-126.