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Hormone Markers of Pituitary Adenomas: An Immunohistochemical Study

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Abstract

Background

The large majority of neoplasms located in the sella turcica are benign pituitary adenomas derived from cells of the adenohypophysis.The incidence of Pituitary adenomas constitutes approximately 10-25% of all intracranial neoplasms.The biology of pituitary adenomas is complex and they can cause a variety of endocrine syndromes and disorders, based on hormone profile secreted by proliferating cells. The classification of pituitary adenomas has changed considerably over time.The first attempt at classification relied on hematoxylin and eosin staining of resected tissues. Modern histologic evaluation utilizes immunohistochemical staining techniques to identify the hormones within adenoma cells.

Aims and Objectives

To study the hormonal subtyping of pituitary adenomas by immunohistochemistry using monoclonal and polyclonal antibodies against the various pituitary hormones and to study the clinical relevance of hormonal markers in Pituitary adenomas.

Methods

An observational study was carried out on 50 surgically resected Pituitary adenomas. They were studied immunohistochemically with Monoclonal/ Polyclonal antisera to 6 anterior pituitary hormones.

Results

24.0% of the case subjects were interpreted as Somatotroph adenomas, 24.0% of the case subjects were interpreted as Gonadotroph adenomas, 22% of the case subjects were interpreted as Corticotroph

adenomas, 14% of the case subjects were interpreted as Lactotroph adenomas, 12% of the case subjects were interpreted as Plurihormonal, 4.0% of the case subjects were interpreted as Mammosomatotroph adenomas.

Conclusion

An integrated evaluation of tumor biopsy by light microscopy and immunohistochemistry is needed to diagnose and classify pituitary adenomas as per latest 2017 WHO classification. It is necessary to know the hormonal profile as certain types of pituitary adenoma are designed as aggressive adenomas in respect to their gender, size and hormonal activity.

Keywords

Pituitary adenoma, monohormonal adenoma, plurihormonal adenoma, atypical adenoma, aggressive adenoma, high-risk adenoma, pituitary neuroendocrine tumor, pituitary carcinoma, immunohistochemical studies.

Introduction

The pituitary gland, or Hypophysis Cerebri is the master gland of the endocrine system because it produces a number of hormones which control the secretions of many other endocrine glands of the body[1]. Thus regulates basic physiological functions incuding growth, reproduction and metabolic homeostasis. The pituitary gland is a reddish-grey, ovoid body. It is situated at the base of the brain.Along with the central nervous system and circulatory systems, the endocrine system co-ordinates and integrates a huge variety of vital body functions. Proliferations of hormone secreting epithelial cells of the anterior lobe can sometimes lead to neoplastic transformation giving rise to Pituitary adenoma.

Pituitary adenomas are a diverse group of tumors arising from the Pituitary gland. The incidence of pituitary adenomas constitutes approximately 10-25% of all intracranial neoplasms[2,3] .The incidence of pituitary adenomas in India varies between 3.8 to 10.5% of all intracranial space occupying lesions [4] .They are the most common tumors of the sellar and suprasellar region[5]. They may occasionally be ectopic[6].The ectopic pituitary adenomas have been reported in sphenoidal sinus.[7]These tumors exhibit a wide range of hormonal and proliferative behavior. They are generally an incidental finding in about most pituitary adenomas are non-invasive and benign although invasive pituitary adenomas are also frequent [8,9].

Etiology of Pituitary adenoma is not well understood. Few cases are associated with known somatic mutations[9]. They also arises as a part of genetic disorders such as Carney complex, Multiple endocrine neoplasia 1, Mccune-Albright syndrome[10,11]. First and most common gene identified that predispose pituitary neoplasia is Pituitary tumor transforming gene (PTTG) seem to play a major role in oncogenesis[12].

The classification of pituitary adenomas has changed considerably over time. The first attempts at classification relied on hematoxylin and eosin staining of resected tissue. Adenomas were categorized as acidophilic, basophilic and chromophobic.[13] This scheme, however, failed to account for clinical manifestations or hormone secretion. 2004 WHO guidelines were more dependent on ultrastructural studies of the secretory granules of the tumor, hence, was more complicated and expensive.[14]

The current 2017 WHO Classification of Pituitary adenomas is defined by hormonal immunohistochemistry of hormones of anterior pituitary and their pituitary specific transcription factors.[9]Therefore, modern histologic evaluation utilizes immunohistochemical staining techniques to

identify the hormones within adenoma cells. The density of secretory granules in a given cell can be determined using Immunohistochemistry. Hence, pituitary adenomas can be further sub-typed into densely granulated and sparsely granulated adenomas.[9,13] This morphological variation is of clinical & prognostic significance.

Pathological classification – Based on the morphology and hormonal immune histochemical assessment

A. Somatotroph adenoma (GH secreting adenoma)

B. Lactotroph adenoma (PRL secreting adenoma)

C. Thyrotroph adenoma (TSH secreting adenoma)

D. Corticotroph adenoma (ACTH secreting adenoma)

E. Gonadotroph adenoma (FSH &/or LH secreting adenoma)

F. Plurihormonal adenoma (More than one hormone secreting adenoma)

G. Null cell adenoma (hormone negative & TF negative)

Plurihormonal adenomas show immunoreactivity of more than one hormone which have different cytogenetic pathway. Therefore, adenomas with combination of GH and PRL, or FSH and LH are not considered as plurihormonal.[9,15,16]

Based on Pituitary adenoma's secretory activity they are further classified clinically as [17]

a. Clinically functioning pituitary adenoma

b. Clinically Non-functioning pituitary adenoma (25-30%)

The aim of the study was to study the hormonal subtyping of pituitary adenomas by immunohistochemistry using monoclonal and polyclonal antibodies against the various pituitary hormones and to study the clinical relevance of hormonal markers in Pituitary adenomas.

Material & Method

The study included patients operated in the Department neurosurgery of Mahatma Gandhi Medical College and Hospital, Jaipur. A total of 50 cases of Pituitary adenomas were included in this study. All the cases were reviewed appropriately and graded using the WHO criteria published on 2016.The study was carried out in the Department of Anatomy in collaboration with Histopathology Department at Mahatma Gandhi Medical college and Hospital, Jaipur. Clinical, biochemical and radiological data were obtained for all the cases.

Inclusion Criteria

Tissues received & diagnosed on H & E as Pituitary adenoma are included in the study.

Exclusion Criteria

a) Biopsies showing extensive infarction.

b) Improperly fixed specimen.

c) Autolysed tissues.

Histology

Tissue sections from formalin-fixed Paraffin-Embedded blocks were stained by routine Haematoxylin and Eosin and observed by Light Microscopy.

Immunohistochemistry

Enzyme linked polymer-based detection method were used for immunohistochemical staining. Specimens of 5 micron thick sections were cut from the formalin-fixed paraffin- embedded blocks and placed on positively charged polylysine coated slides.

Positive Control

Parallel positive control sections were processed with each set of immunostaining.

Negative Control

Sections untreated with primary antibody were considered as negative controls for each set of slides.

Antibody Panel

1. GH - Monoclonal, Rabbit, Clone- EP267, Company - Dako, Ready to use.
2. PRL - Monoclonal, Rabbit, Clone- EP193, Company - Dako, Ready to use
3. TSH - Monoclonal, Rabbit, Clone- EP254 , Company - Dako, Ready to use
4. ATCH - Polyclonal, Rabbit, Company – Dako, Ready to use
5. LH - Monoclonal, Rabbit, Clone- C93 , Company - Dako, (1:50 dilution)
6. FSH - Monoclonal, Rabbit, Clone- EP257, Company - Dako, Ready to use

Reagents Used

1. Diva decloaker 20X- (1:20 dilution) . It is used with Biocare's digital electric pressure cooker (Decloaking chamber).
2. TBS Wash buffer, 40X- Mix 1 part of concentrated buffer to 39 parts of deionized water (1:40 dilution). pH should be 7.7+/-0.1 at 25 degree.
3. Background sniper
4. MACH 2 Universal HRP-Polymer Detection
5. Betazoid DAB chromogen-3,3' diaminobenzidine (DAB) is widely used chromogen for immunohistochemical staining.

(Mix 1 drop of DAB chromogen per 1 ml of DAB substrate buffer. The DAB working solution is stable for 5 days when stored at 2-8 degrees.)

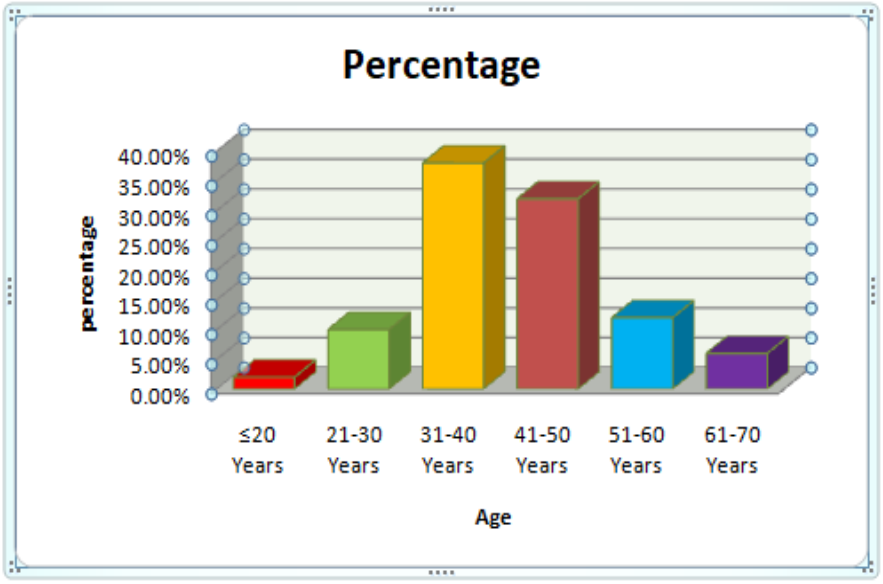
- Standard Operative Procedure for immunohistochemistry was performed.
- Immunostaining was evaluated in the fields consisting of regions of the tumour having the greatest number of immunoreactive cells, as assessed qualitatively at low-power examination.
- Cytoplasmic staining, in > 10% of cells was regarded as positive.

Results & Observation

Table 1: Distribution of the Participants in Terms of Age (n = 50)

Age	Frequency	Percentage
≤20 Years	1	2.00%
21-30 Years	5	10.00%
31-40 Years	19	38.00%
41-50 Years	16	32.00%
51-60 Years	6	12.00%
61-70 Years	3	6.00%
Total	50	100%

2% of the participants had Age: ≤20 Years. 10.0% of the participants had Age: 21-30 Years. 38.0% of the participants had Age: 31-40 Years. 32.0% of the participants had Age: 41-50 Years. 12.0% of the participants had Age: 51-60 Years. 6.0% of the participants had Age: 61-70 Years.

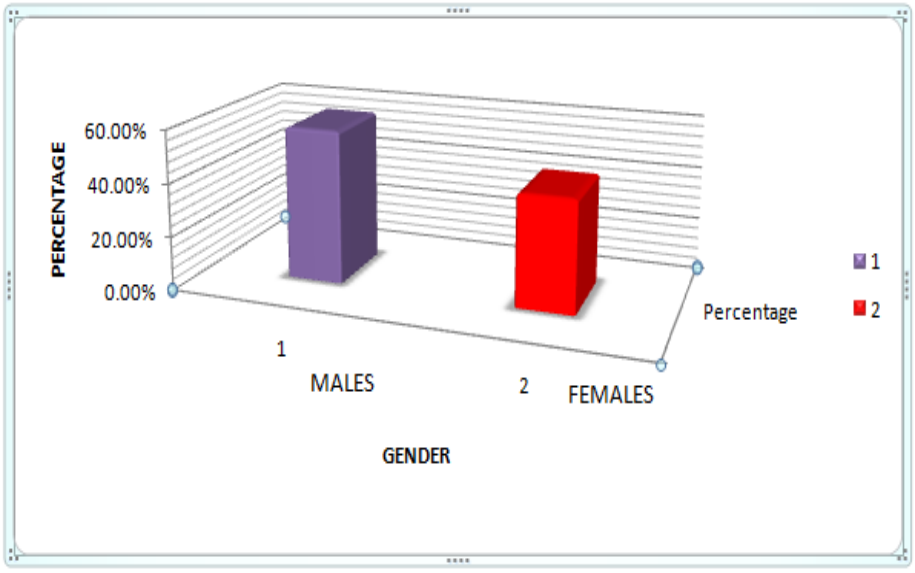


Graph 1: Distribution of the Participants in Terms of Age

Table 2: Distribution of the Participants in Terms of Gender (n = 50)

Gender	Frequency	Percentage
Male	29	58.00%
Female	21	42.00%

58.00% of the participants were Male. 42.00% of the participants were Female.



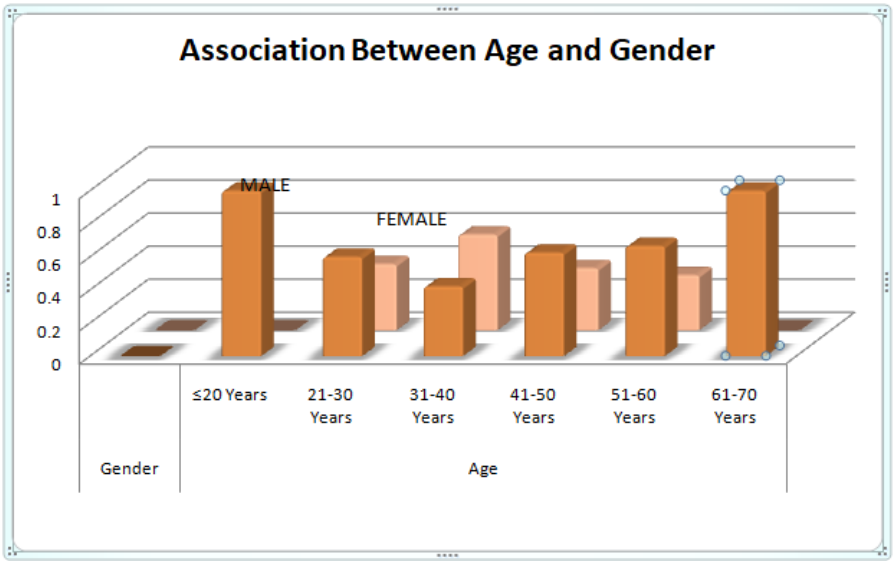
Graph 2: Distribution of the Participants in Terms of Gender

Table 3: Association between Age and Gender (n = 50)

Gender	Age							Fisher's Exact Test	
	≤20 Years	21-30 Years	31-40 Years	41-50 Years	51-60 Years	61-70 Years	Total	χ2	P Value
Male	1 (100.0%)	3 (60.0%)	8 (42.10%)	10 (62.5%)	4 (66.6%)	3 (100.0%)	29 (58.00%)	2.0525	0.2516
Female	0 (0.0%)	2 (40.0%)	11 (57.89%)	6 (37.5%)	2 (33.3%)	0 (0.0%)	21 (42.00%)		
Total	1 (100.0%)	5 (100.0%)	19(100.0%)	16 (100.0%)	6 (100.0%)	3(100.0%)	50 (100.0%)		

Fisher's exact test was used to explore the association between 'Age' and 'Gender' as more than 20% of the total number of cells had an expected count of less than 5.

52.0 % of the test subjects were female up to 40 years and 68% of the subjects were male for age above 40 years. 61.9 % of female subjects were below 40 years of age and 58.6% of male subjects were above 40 years of age.



Graph 3: Association between Age and Gender

Table 4: Distribution of the study subjects in terms of Hormonal Activity (n = 50)

Hormonal Activity	Frequency	Percentage
Active	37	74.0%
Silent	13	26.0%
Total	50	100.0%

74.0% of the participants were Clinical Functioning. 26.0% of the participants were Clinically Silent.

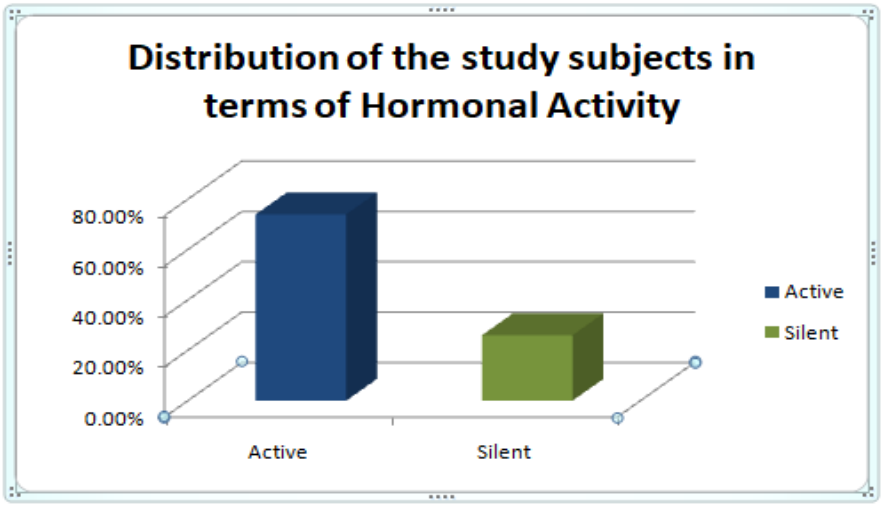


Table 5: Distribution of Clinically functioning study subjects (n = 37)

Clinical Manifestation	Frequency	Percentage
Acromegaly	14	37.83%
Cushing Disease	9	24.32%
Hyperprolactinemia	6	16.21%
Recurrent	5	13.51%
FSH Secreting	2	5.4%
Hyperthyroidism	1	2.7%
Total	37	100.0%

A cromegaly was the most commonly encountered manifestation in clinically functional adenomas seen in 37.83% of study subjects followed by Cushing disease in 24.32% study subjects.

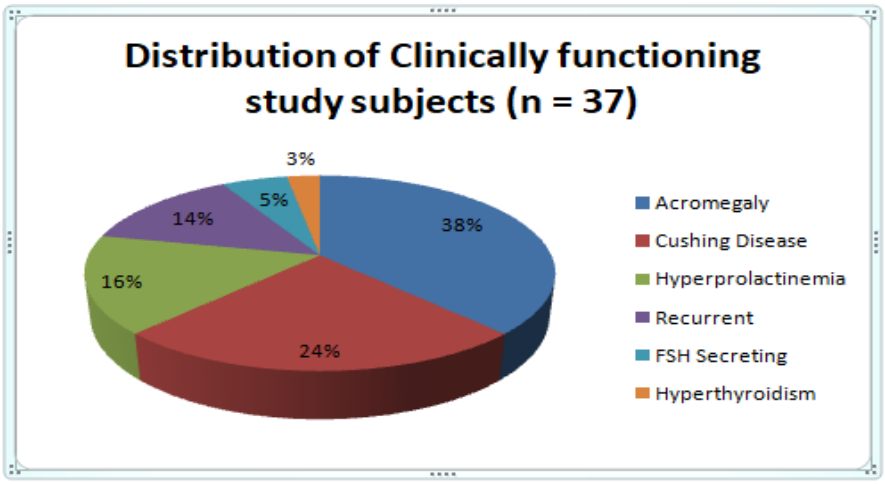


Table 6: Distribution of the Study subjects in Terms of IHC Interpretation

Interpretation	Frequency	Percentage
Somatotroph	12	24.0%
Gonadotroph	12	24.0%
Corticotroph	11	22.0%
Lactotroph	7	14.0%
Plurihormonal	6	12.0%
Mammosomatotroph	2	4.0%
Total	50	100.0%

26.0% of the case subjects were interpreted as Somatotroph. 26.0% of the case subjects were interpreted as Gonadotroph. 20.0% of the case subjects were interpreted as Corticotroph.. 12.0% of the case subjects were interpreted as Lactotroph. 10.0% of the case subjects were interpreted as Plurihormonal. 6.0% of the case subjects were interpreted as Mammosomatotroph.

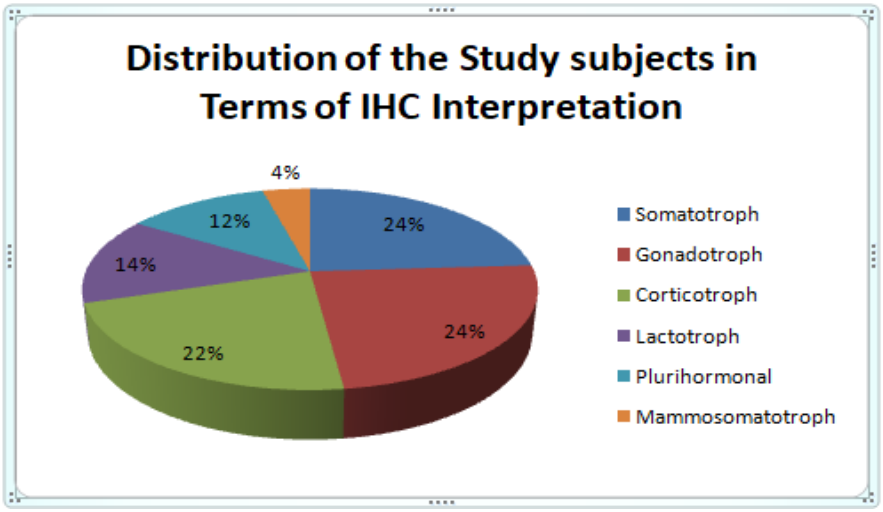
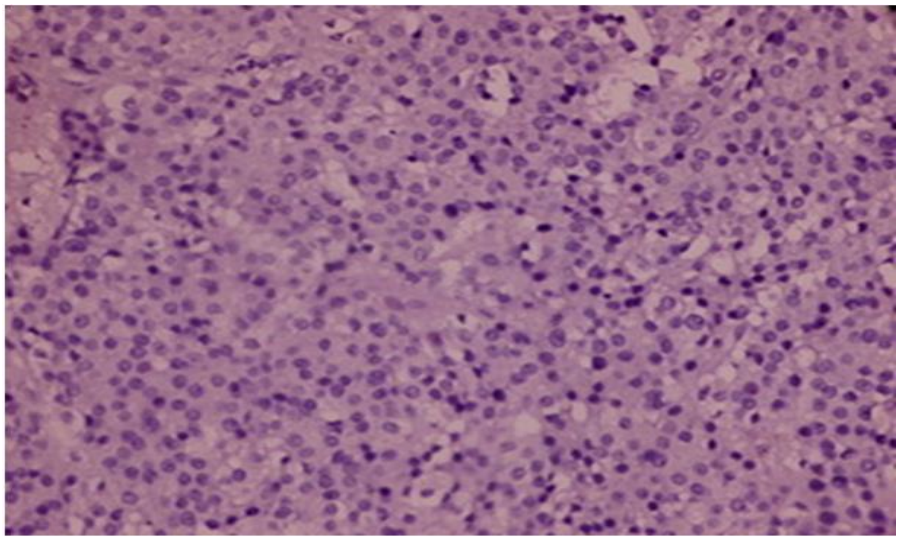


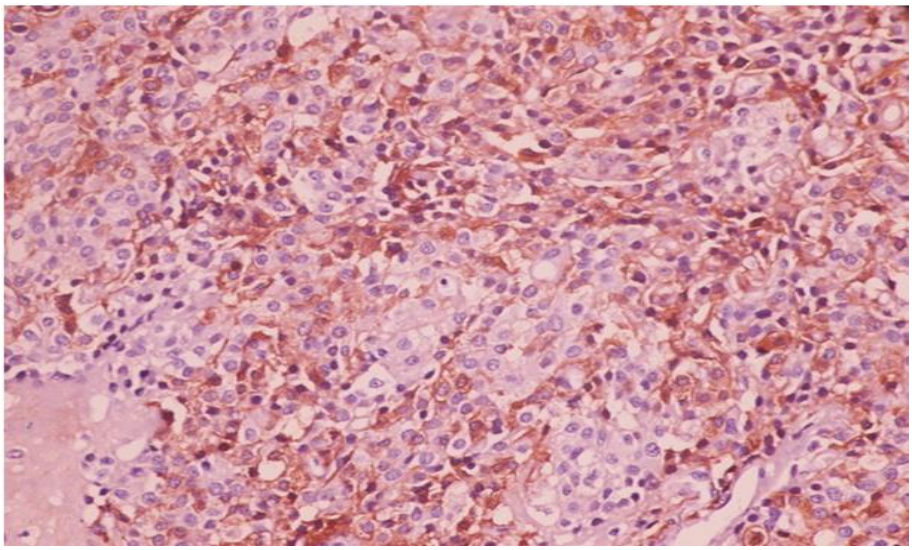
Table 7: Association between Gender and IHC Interepretation (n = 50)

IHC Interepretation	Gender			P Value
	Male	Female	Total	
Somatotroph	6 (20.68%)	6 (28.57 %)	12 (24.0%)	
Gonadotroph	7 (24.13%)	5(23.80 %)	12 (24.0%)	
Corticotroph	7 (24.13%)	4 (19.04 %)	11 (22.0%)	
Lactotroph	5 (17.24%)	2 (9.52 %)	7 (14.0%)	0.946
Plurihormonal	3 (6.89%)	3 (9.52 %)	6 (12.0%)	
Mammosomatotroph	1 (3.44 %)	1 (4.76 %)	2 (4.0%)	
Total	29 (100.0%)	21 (100.0 %)	50 (100.0%)	

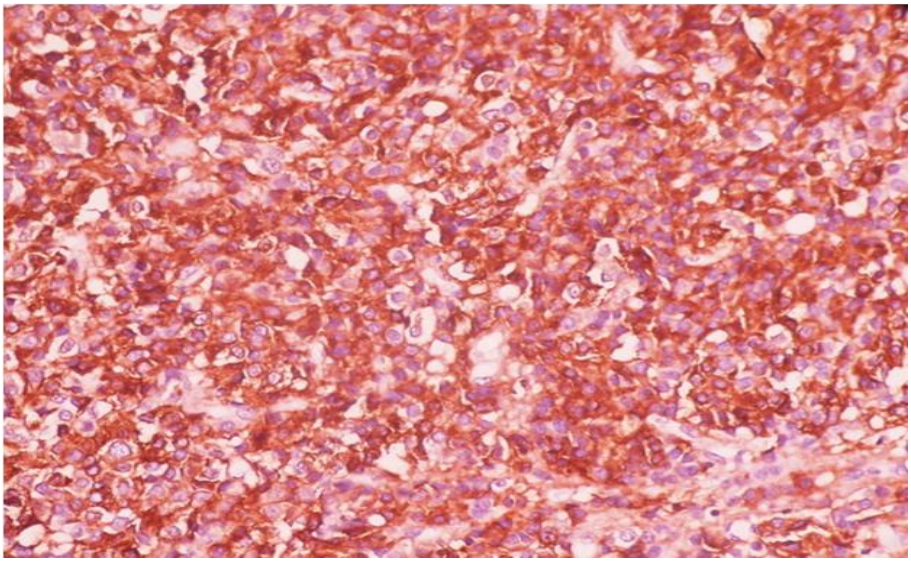
Lactotroph, Gonadotroph and Corticotrophs had higher frequency in male. Whereas somatotrophs, plurihormal adenoma and Mammosomatotrophs were in equal proportion in male and female.



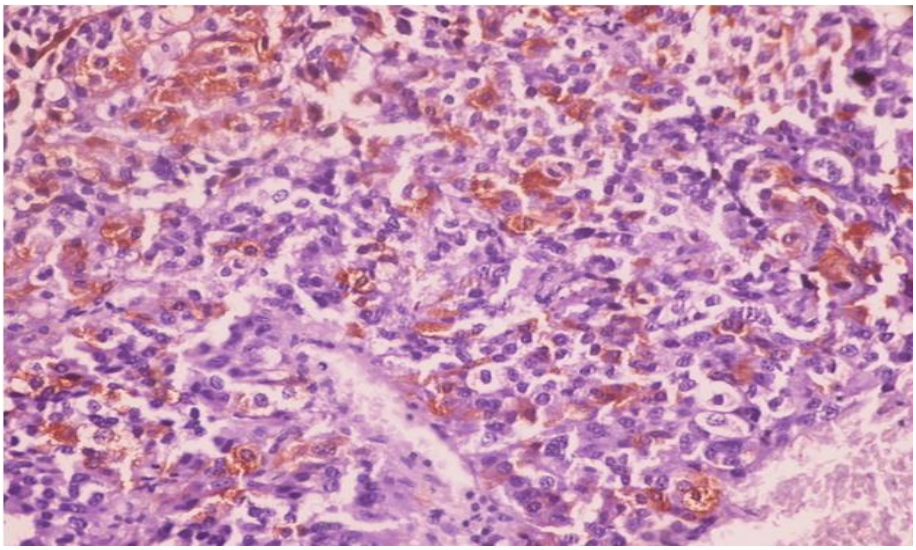
Pituitary Adenoma H & E STAIN 40 ×



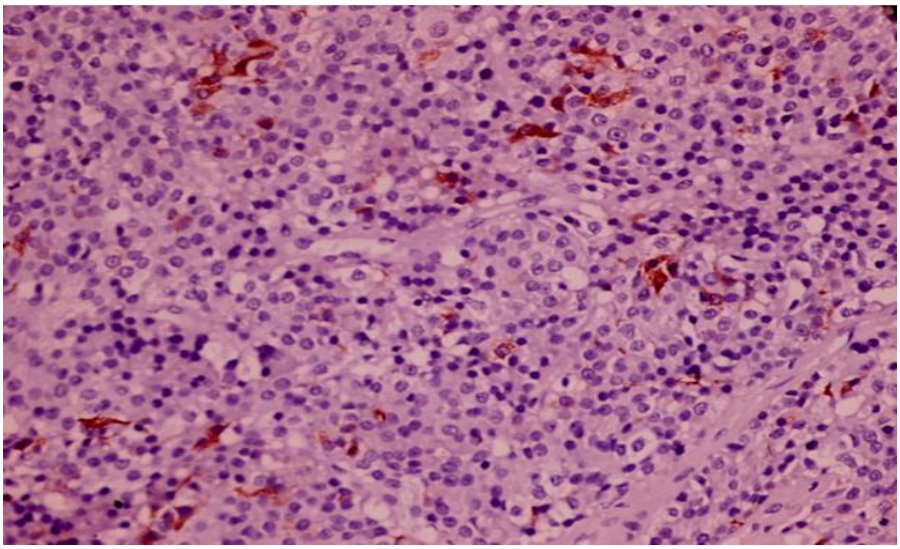
Somatotroph Adenoma GH 40 ×



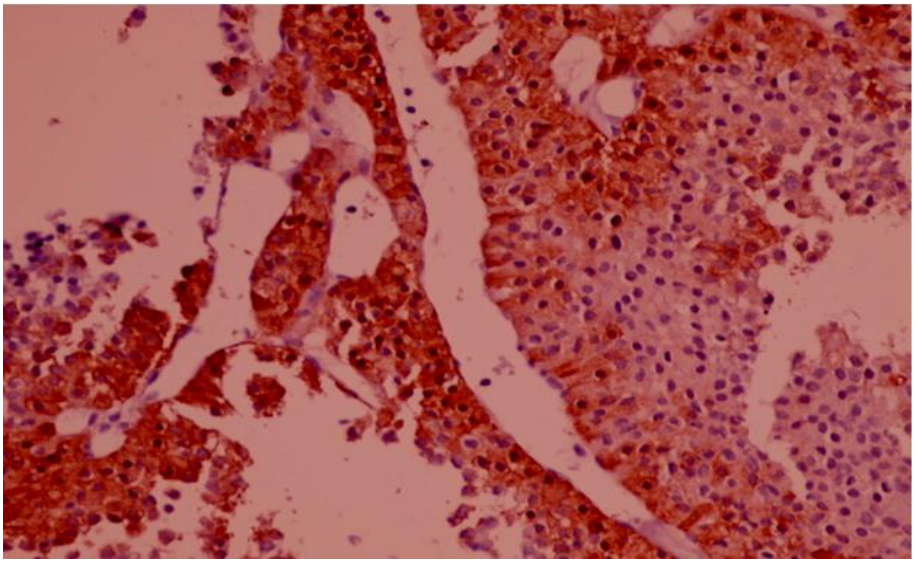
PRL Adenoma, 40x



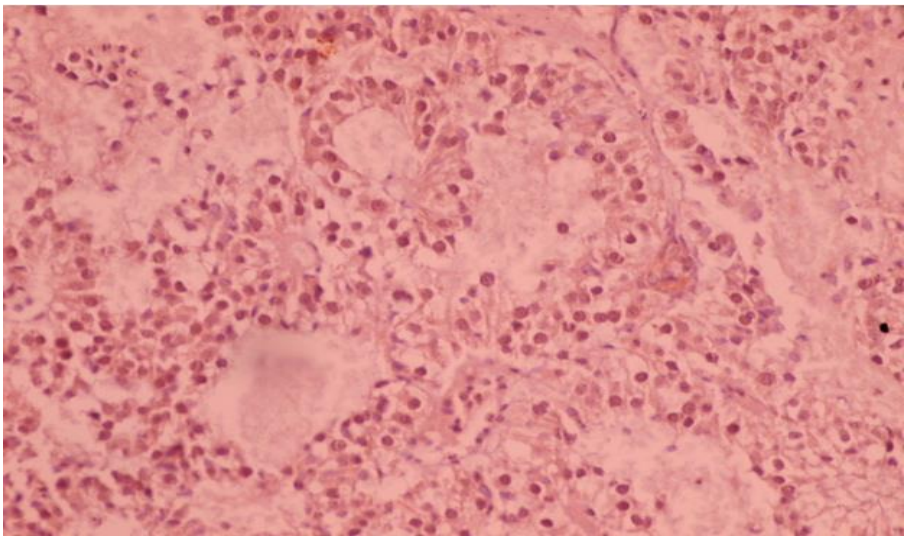
Corticotroph Adenoma ACTH 40x



Plurihormonal Adenoma TSH 40x



Gonadotroph Adenoma FSH 40x



Gonadotroph Adenoma LH 40x

Discussion

Pituitary adenoma arises from one pituitary cell type or more than one cell type and is the most common tumor of the sellar region. Adenomas lack reticulin supported acinar structure of the gland, growth of tumor compresses the adjacent normal gland and its acinar structure and reticulin microskeleton that results in a surgical pseudocapsule which encases the adenoma separating it from the normal gland.[18] Clinical symptoms of pituitary adenomas depend upon their hormonal activity, size and immunohistological subtype.

Tinctorial classification based on various histochemical stains has been used for a long time in morphological studies of pituitary adenomas[19,20].This simple classification is insufficient to cover the complex clinicopathologic aspects of this type of tumor. Recent advances in immunocytochemistry have provided a highly specific and sensitive tool to demonstrate the presence of hormones in tissue section[21] .

In our study male predominance of 58% was observed in our study of 50 cases, however, the biggest study conducted for gender related occurrence of pituitary adenoma by T Mindermann and C B Wilson concluded that frequency of pituitary adenoma differ greatly in their male to female ratio.[19]

Clinically functioning adenomas are relatively easy to diagnose due to hypersecretion of hormones whereas non-functioning adenomas are difficult to diagnose as they do not cause any syndrome.[21]Non functioning adenomas of large size produce compression effects on the nearby structures whereas smaller silent adenomas remain undiagnosed through the life.

Prevalence of silent adenomas in our study was 24% of the total cases. Duc to large number of undiagnosed cases of silent adenomas there is a large variation in past studies done. The most recent study of Juliana Drummond et al in 2019 gave a range of 22-54% of incidences of silent adenomas.[21]

Only one case of Silent adenoma was below 40 years of age in our study, indicating an increase in incidence of silent adenomas with age. Georgia Ntali and John A.Wass did an extensive literature review and

published a retrospective study in 2017[22] .Their result yielded the peak occurrence of clinically non-functioning adenomas from fourth to eighth decade, thus supporting our study.

No hormone-immunonegative adenoma was encountered in our study.Somatotroph adenomas and Gonadotroph adenomas were the most common type of Pituitary adenoma reported in our study. Study done by Jiayu Liu et al in 2020 also showed most cases of gonadotrophs[23]. No study in our review of literature had somatotroph as most common adenoma.

Total 12 somatotrophs (24%) were diagnosed in our study. Somatotroph adenoma are adenomas which show immunoreactivity with GH. They are often hormonally active and leading to GH excess and tend to occur in older age groups.Somatotroph adenomas in our study had an equal ratio among male and female and was in concordance with study by Howlett, T.A., Willis, D., Walker, et al which also had an equal male female ratio[24].

Twelve Gonadotrophs were seen our study (24%). Gonadotroph adenomas produce either FSH, LH or both immunohistochemically. They are believed to arise spontaneously. Most gonadotrophs produce symptoms related to mass effects caused by tumor. Among premenopausal women menstrual disorders can be seen. Gonadotrophs may be present with impotency in man[9,24].

Our study had a male female ratio of 1.4:1. Gonadotroph adenoma have a slight male preponderance[9,25] . Seven cases showed immunoreactivity with FSH, two cases with both FSH and LH. One case showed immunoreactivity with LH.

seven Lactotroph adenomas (14%) were seen in our study. Lactotroph adenomas show immunohistochemical positivity with Prolactin hormone marker. Lactotroph adenomas are frequent cause of ovulatory disorders among women and erectile dysfunction in men[9].Their pathogenesis is relatively unknown. Although experiments show role of estrogen in development of lactotroph adenoma[26] .They are also relatively common during pregnancy.

Two cases in our study showed diffuse positivity of for both Prolactin and Growth hormone as were interpreted as Mammosomatotroph and were further advised for ultrastructural studies. Both cases were less than 35 years of age, frequency ofMammosomatotrophs is more among younger age group as compared to somatotrophs.[9,27]

Six Plurihormonal adenomas (12%) were seen in our study. Plurihormonal adenomas are rare forms of pituitary adenoma with unusual immunohistochemical combination which cannot be explained by cytodifferentiation. Plurihormonal adenomas can present with acromegaly, hyperprolactinemia, hyperthyroidism or cushing disease. They can also be clinically silent[28,29,30].Plurihormonal adenoma in our study presented with cushing disease, hyperprolacteniemia and hyperthyroidism.

All plurihormonal adenomas in our study showed immunoreactivity with TSH. Three of the four TSH secreting adenomas co expressed with GH and PRL, correlating with the previous study by Kirkman MA et al which had 84% of TSH secreting adenoma co expressing GH and PR[31].

No independent thyrotroph was reported in our study. Thyrotrophs are the rare type of pituitary adenomas. But, their diagnosis is very useful as failure to recognize it may lead to thyroid ablation causing expansion of pituitary volume and thus leading to invasive disease[9].

Eleven cases (22.0%) in our study were Corticotroph adenomas. Corticotroph adenoma expresses ACTH on immunohistochemistry.They arise from TPIT-1 lineage hypophyseal cells.[9] Most corticotrophs are biochemically and clinically functioning, producing Cushing syndrome. Cushing syndrome was first described by Harvey Cushing in 1912[35].

Conclusion

Pituitary adenoma are frequently encountered tumours, silent pituitary adenomas represent a challenging group of tumors. Our study signifies the importance of immunohistochemistry markers for diagnosing clinically silent adenomas. An integrated evaluation of tumor biopsy by light microscopy and immunohistochemistry is needed to diagnose and classify pituitary adenomas as per latest 2017 WHO classification. From clinical point of view, it is necessary to know the hormonal profile as certain types of pituitary adenoma.We recommend a multidisciplinary approach necessary for diagnosis and optimal management of pituitary adenoma patients.

References

1. B.D Chaurasia's Human Anatomy (Head & Neck) , 7th edition CBS Publishers & Distributors PVt Ltd (3: 204)
2. Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, McCutcheon IE (August 2004). "The prevalence of pituitary adenomas: a systematic review". *Cancer*. 101 (3): 613–9.
3. Kovacs K,Scheithauer BW,Horvath E, Lloyd RV.The World Health Organization classification of adenohypophysial neoplasms. A proposed five-tier scheme. Cancer 1996;78:502-10.
4. Roldan-Valadez E, Garcia-Ulloa AC, Gonzalez-Gutierrez O, Martinez-Lopez M. 3D volumetry comparison using 3T magnetic resonance imaging between normal and adenoma-containing pituitary glands. Neurol India 2011; 59:696-699.
5. Al-Brahim NY, Asa SL. My approach to pathology of the pituitary gland. *J Clin Pathol* 2006;59(12):1245-1253. doi:10.1136/jcp.2005.031187.
6. Coire CI, Horvath E, Kovacs K, Smyth HS, Ezzat S 1997 Cushing's syndrome from an ectopic pituitary adenoma with peliosis: a histological, immunohistochemical and ultrastructural study and review of the literature. Endocr Pathol 8:65–74.
7. Thompson, L. D., Seethala, R. R., & Müller, S. (2012). Ectopic sphenoid sinus pituitary adenoma (ESSPA) with normal anterior pituitary gland: a clinicopathologic and immunophenotypic study of 32 cases with a comprehensive review of the english literature. *Head and neck pathology*, *6*(1), 75–100.
8. Pamela U. Freda, Albert M. Beckers, Laurence Katznelson, Mark E. Molitch, Victor M. Montori, Kalmon D. Post, Mary Lee Vance, Pituitary Incidentaloma: An Endocrine Society Clinical Practice Guideline, *The Journal of Clinical Endocrinology & Metabolism*, Volume 96, Issue 4, 1 April 2011, Pages 894–904.
9. Lopes, M. (2017). The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. Acta Neuropathologica. 134. 1-15. 10.1007/s00401-017-1769-8.
10. Schernthaner-Reiter MH, Trivellin G, Stratakis CA. MEN1, MEN4, and Carney Complex: Pathology and Molecular Genetics. *Neuroendocrinology*. 2016;103(1):18-31. doi:10.1159/000371819.
11. Stergiopoulos SG, Abu-Asab MS, Tsokos M, Stratakis CA. Pituitary pathology in Carney

complex patients. *Pituitary*. 2004;7(2):73-82. doi:10.1007/s11102-005-5348-y.

12. Tfelt-Hansen J, Kanuparthi D, Chattopadhyay N. The emerging role of pituitary tumor transforming gene in tumorigenesis. *Clin Med Res*. 2006;4(2):130-137. doi:10.3121/cmr.4.2.130.
13. Ironside JW (August 2003). "Best Practice No 172: pituitary gland pathology". *Journal of Clinical Pathology*. 56 (8): 561–8.
14. Lloyd RV, Kovacs K, Young Jr WF, Farrell WE, Asa SL. Pituitary tumours: Introduction. DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors In: WHO Classification of Tumours. Pathology and Genetics. Tumors of Endocrine Organs. 3rd ed. Lyon: IARC Publications; 2004.
15. Erickson D, Scheithauer B, Atkinson J, et al. Silent subtype 3 pituitary adenoma: a clinicopathologic analysis of the Mayo Clinic experience. *Clin Endocrinol (Oxf)*. 2009;71(1):92-99.
16. E. Horvath, K. Kovacs, H. S. Smyth, D. W. Killinger, B. W. Scheithauer, R. Randall, E. R. Laws, JR., W. Singer, A Novel Type of Pituitary Adenoma: Morphological Features and Clinical Correlations, *The Journal of Clinical Endocrinology & Metabolism*, Volume 66, Issue 6, 1 June 1988, Pages 1111–1118.
17. Rosai, J., Ackerman, L. V. 1., &Rosai, J. (2018). *Rosai and Ackerman's surgical pathology* (11th ed.).
18. Taylor DG, Jane JA, Oldfield EH. Resection of pituitary macroadenomas via the pseudocapsule along the posterior tumor margin: a cohort study and technical note. *J Neurosurg*. 2018;128(2):422-428.
19. Kernohan JW, Sayre GP. Tumors of the pituitary gland and infundibulum. Atlas of Tumor Pathology, Section X, Fascicle 36.Washington, DC: Armed Forces Institute of Pathology, 1956.
20. Berry RG, Caplan HJ. An overview of pituitary tumors. *Ann Clin Lab Sci* 1979; 9:94-102.
21. Mukai K, Rosai J. Applications of immunoperoxidase techniques in surgical pathology. In: Fenoglio CM, Wolff M, eds. Progress in Surgical Pathol, vol. I . New York: Masson Publishing, 1980 15-49.
22. Mindermann T, Wilson CB. Age-related and gender-related occurrence of pituitary adenomas [published correction appears in Clin Endocrinol (Oxf) 1994 Nov;41(5):700]. *Clin Endocrinol (Oxf)*. 1994;41(3):359-364.
23. Drummond J, Roncaroli F, Grossman AB, Korbonits M. Clinical and Pathological Aspects of Silent Pituitary Adenomas. *J Clin Endocrinol Metab*. 2019;104(7):2473-2489. doi:10.1210/jc.2018-00688.
24. E. Horvath, K. Kovacs, H. S. Smyth, D. W. Killinger, B. W. Scheithauer, R. Randall, E. R. Laws, JR., W. Singer, A Novel Type of Pituitary Adenoma: Morphological Features and Clinical Correlations, *The Journal of Clinical Endocrinology & Metabolism*, Volume 66, Issue 6, 1 June 1988, Pages 1111–1118.
25. Ntali, Georgia & Wass, John. (2018). Epidemiology, clinical presentation and diagnosis of non-functioning pituitary adenomas. Pituitary. 21. 10.1007/s11102-018-0869-3.
26. Liu, J., He, Y., Zhang, X., Yan, X., & Huang, Y. (2020). Clinicopathological analysis of 250 cases of pituitary adenoma under the new WHO classification. Oncology Letters, 19, 1890-1898.
27. Howlett, T.A., Willis, D., Walker, G., Wass, J.A.H., Trainer, P.J. and (2013), Control of growth

hormone and IGF1 in patients with acromegaly in the UK: responses to medical treatment with somatostatin analogues and dopamine agonists. Clin Endocrinol, 79: 689-699.

28. Georgia Ntali, Cristina Capatina, Ashley Grossman, Niki Karavitaki, Functioning Gonadotroph Adenomas, *The Journal of Clinical Endocrinology & Metabolism*, Volume 99, Issue 12, December 2014, Pages 4423–4433.
29. Heaney AP, Fernando M, Melmed S. Functional role of estrogen in pituitary tumor pathogenesis. *J Clin Invest.* 2002;109(2):277-283. doi:10.1172/ JCI14264.
30. Asa SL, Ezzat S. The pathogenesis of pituitary tumors. Annu Rev Pathol. 2009;4:97-126.
31. 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. *Ultrastruct Pathol*. 2005;29(6):511-524.
32. Pawlikowski M, Kunert-Radek J, Radek M (2010) Plurihormonality of pituitary adenomas in light of immunohistochemical studies. Endokrynol Pol 61:63–66
33. Erickson D, Scheithauer B, Atkinson J, et al. Silent subtype 3 pituitary adenoma: a clinicopathologic analysis of the Mayo Clinic experience. *Clin Endocrinol (Oxf)*. 2009;71(1):92-99.
34. Kirkman MA, Jaunmuktane Z, Brandner S, Khan AA, Powell M, Baldeweg SE. Active and silent thyroid-stimulating hormone-expressing pituitary adenomas: presenting symptoms, treatment, outcomes, and recurrence. *World Neurosurg*. 2014;82(6):1224-1231.
35. Zada, G., Carmichael, J. D., & Selman, W. R. (2015). Introduction: Cushing's disease: a century of evolving diagnostics and therapeutics for an often elusive entity, *Neurosurgical Focus FOC*, *38*(2), E1.
36. Kernohan JW, Sayre GP. Tumors of the pituitary gland and infundibulum. Atlas of Tumor Pathology, Section X, Fascicle 36.
37. Washington, DC: Armed Forces Institute of Pathology, 1956.
38. Berry RG, Caplan HJ. An overview of pituitary tumors. *AnnClin Lab Sci* 1979; 9:94-102.