EXPRESSION OF Pit-1, SF-1, Ki-67 LABELLING INDEX IN CORRELATION WITH IMMUNOHISTOCHEMICAL EXPRESSION OF HORMONAL MARKERS IN PITUITARY ADENOMAS

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

PATHOLOGY

JULY 2022 **Dr. ISMETARA BEGAM**

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DECLARATION

I hereby declare that the thesis titled **"EXPRESSION OF Pit-1, SF-1, Ki-67 LABELLING INDEX IN CORRELATION WITH IMMUNOHISTOCHEMICAL EXPRESSION OF HORMONAL MARKERS IN PITUITARY ADENOMAS"** embodies the original work carried out by the undersigned in All India Institute of Medical Science, Jodhpur.

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CERTIFICATE

This is to certify that the thesis entitled **"EXPRESSION OF Pit-1, SF-1, Ki-67 LABELLING INDEX IN CORRELATION WITH IMMUNOHISTOCHEMICAL EXPRESSION OF HORMONAL MARKERS IN PITUITARY ADENOMAS"** is the bonafide work of **Dr. ISMETARA BEGAM** carried out under our guidance and supervision, in the Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur.

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Acknowledgments

I would like to dedicate this thesis to my dearest and beloved grandmother, "***BHALOMA***" whose sudden demise has left a huge void in my life.

As I sit down to write this acknowledgement section of my thesis, so many faces and memories flood my mind, without whom, this project would have been impossible .

Firstly, I would like to start my vote of thanks by singing my school, South Point High School's assembly prayer, a song without which my day's journey is incomplete:

"Dear God,
Thank you for everything;
Bless me and help me to be good,
And to do my studies well"

Indeed, I find myself extremely privileged and grateful to have ***Dr. Poonam Abhay Elhence***, Professor and Head of Department, Department of Pathology and Lab Medicine as my supervisor. This project has been possible only because of her expert guidance, invaluable time, support and motivation. She instilled in me the sense of perseverance and honest observation, and her explanation of all the slides was a beautiful smooth narrative. I could have never completed this dissertation without her and shall always be indebted to her for this holistic experience that I received under her umbrella.

I express my sincere thanks to my co-guides ***Dr. Sudeep Khera***, Associate Professor, Department of Pathology and Lab Medicine, AIIMS Jodhpur and ***Dr. Deepak Vedant***, Assistant Professor, Department of Pathology and Lab Medicine, AIIMS Jodhpur. Without their constant support, inspiration, valuable time and guidance I could have never finished this project.

I am extremely thankful to ***Dr. Madhukar Mittal***, Additional Professor, Department of Endocrinology and Metabolism, AIIMS Jodhpur and ***Dr. Mayank Garg***, Associate Professor, Department of Neurosurgery, AIIMS Jodhpur for their constant support and help throughout my work. I cannot thank them enough for their invaluable efforts.

I would also like to thank all the faculties of the Department of Pathology and Lab Medicine, AIIMS Jodhpur for their constant support.

I would like to express my sincere gratitude to ***Dr. Akhil Dhanesh Goyal***, Assistant Professor, Department of Community and Family Medicine, AIIMS Jodhpur for helping me with the statistical aspect of my study.

I find myself lucky to have batchmates like ***Dr. Anju G*** and ***Dr. Kartik Jain***, who were also there for me whenever I needed any help.

All my beloved juniors and amazing seniors deserve my heartiest gratitude for their selfless help, suggestion and constant motivation they provided round the clock during the study.

I cannot express how grateful I am to my husband, ***Dr. Ashadur Zamal*** for helping me and supporting me in all aspects of my life.

I am extremely thankful to all the Technical staff, especially ***Mr. Neeraj, Mr. Prem Shankar and Mrs. Meenakshi Upadhyay*** for helping me in the practical aspect of thesis.

Last but not the least, I would like to thank my parents, ***Mr. Osman Gani*** and ***Mrs. Nurjahan Begam*** and my in-laws for their unconditional love and support.

My special acknowledgement goes to all those who directly and indirectly made it possible to complete my MD thesis.

Last but not the least, I would like to thank ***God Almighty*** for giving me the strength and perseverance to complete this study satisfactorily. Without his blessings, nothing is possible.

Dr. Ismetara Begam

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SYNOPSIS

Pituitary adenoma (PA) is the most common benign tumor of the anterior pituitary gland. Fifty diagnosed cases of PA were included in this study. The present study assessed the immunoexpression of the transcription factors (Pit-1 and SF-1) along with the proliferating marker Ki-67 LI (MIB1) in correlation with the hormones secreted by the anterior pituitary gland (GH, PRL, β-FSH, β-LH, β-FSH and ACTH).

Additional IHC for CAM 5.2, Pan-CK, Chromogranin A, Synaptophysin, TTF-1 and p53 was done, wherever required.

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AIP	Aryl hydrocarbon receptor interacting protein
AMP	Adenosine monophosphate
ASCA	Acidophil stem cell adenoma
BMP	Bone morphogenetic protein
CA	Corticotroph adenoma
CAM5.2	Low molecular weight cytokeratin
CDK	Cyclin dependent kinase
CK	Cytokeratin
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DALY	Disability-adjusted life year
DGLA	Densely granulated lactotroph adenoma
DGSA	Densely granulated somatotroph adenoma
DNA	Deoxyribonucleic acid
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
FSH	Follicle stimulating hormone

GA	Gonadotroph adenoma
GH	Growth hormone
GNAS	Guanine nucleotide binding protein, alpha stimulating activity polypeptide
Gs-A	G protein alpha subunit
HRCT	High resolution computed tomography
IGF1	Insulin like growth factor 1
IHC	Immunohistochemistry
LA	Lactotroph adenoma
LH	Luteinizing hormone
MEN	Multiple endocrine neoplasia
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
NCA	Null cell adenoma
NFPA	Non functioning pituitary adenoma
PA	Pituitary adenoma
PAS	Periodic Acid Schiff
PIT 1	POU domain, class 1,transcription factor 1
PitNET	Pituitary neuroendocrine tumor
POMC	Proopiomelanocortin
PRL	Prolactin
QALY	Quality adjusted life years

RNA	Ribonucleic acid
SA	Somatotrophic adenoma
SEER	Surveillance, Epidemiology and End Results
SF-1	Steroidogenic factor 1
SGLA	Sparsely granulated lactotroph adenoma
SGSA	Sparsely granulated somatotroph adenoma
SPA	Silent pituitary adenoma
SSTR2	Somatostatin receptor 2
TA	Thyrotroph adenoma
TPIT	T-box transcription factor
TSA	Trans sphenoidal adenectomy
TSH	Thyroid stimulating hormone
TVDT	Tumor volume doubling time
WHO	World Health Organization

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“If the Human Brain Were So Simple That We Could Understand It,

We Would Be So Simple That We Couldn’t” - E M. Pugh


INTRODUCTION

INTRODUCTION

In the human body, the action of hormones is similar to that of a well-orchestrated symphony performed by a team of musicians. It was in the year 1931 that Sir Walter Langdon-Brown first wrote "the pituitary is the leader in the endocrine orchestra" [(1)]. This pea-sized small gland perched in the hypophyseal fossa integrates signals from the hypothalamus, to which it is connected by the pituitary stalk and regulates the activity of the thyroid gland, the adrenal gland and the gonads.

Pituitary tumors have a long and fascinating history. A recent DNA examination from the teeth of an Irish patient with gigantism living from 1761 to 1783 and housed at the Hunterian Museum in London, revealed the same mutation in the AIP (Aryl hydrocarbon receptor interacting protein) gene present in 4 families with pituitary tumors from Northern Ireland [(2)]. The skull of the index patient was examined in 1909 and found to have an enlarged pituitary fossa. In 1886, Pierre Marie, a French neurologist was the first to analyze two patients with symptoms of gigantism and created the term "acromegaly". He also postulated the pathology of acromegaly to be arising from the pituitary gland. Despite the fact that gigantism and acromegaly have been described since ancient times, the link between a pituitary adenoma (PA) and clinical acromegaly and gigantism was not recognized until the second half of the nineteenth century [(3)].

PA is an umbrella term given to the benign neoplastic proliferation of anterior pituitary hormone producing cells. Pituitary tumors constitute almost 15% of central nervous system tumors. Among these, pituitary adenoma or pituitary neuroendocrine tumor (PitNET) tumors constitute 10% of all CNS tumors and incidental pituitary tumors are found in approximately 10% of autopsies [(4).] The tumors are typically benign, but some can be aggressive and invade adjacent structures. Pituitary carcinomas are rare accounting for 0.1-0.2% of all pituitary endocrine tumors [(5)].

The worldwide prevalence rate is around 16.7% and it increases with age, with the peak age of diagnosis being between 30 and 60 years in men[(6)].

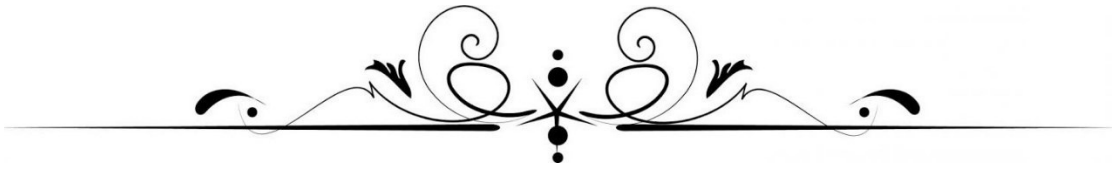
These slow-growing neuroendocrine tumors cause symptoms due to excess hormone release, local space-occupying effects or both. Of all the PAs discovered during lifetime, the prevalence of clinical significance is 1:1000 and from the ones that go to surgery, 10% manifest an atypical, neuroendocrine tumor-like behavior.

Prior to 2017, PAs were divided according to their staining properties into chromophobic, acidophilic and basophilic adenomas. This classification is only of limited use since it does not shed light upon the adenoma cells' endocrine function and the cell type or lineage from which the tumor arises. Use of Electron Microscopy and Immunohistology, resulted in a new PA categorization The 2017 edition of the WHO Classification of Tumors of Endocrine Organs has put much importance on the immunohistological classification of PAs [(7)].

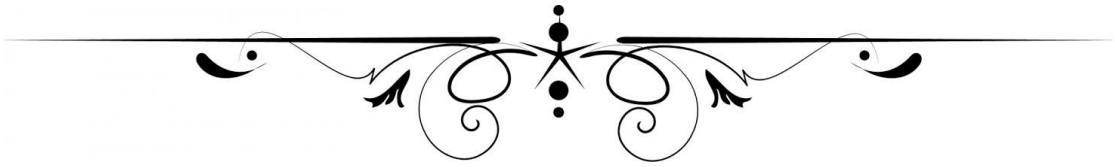
Immunohistochemistry for transcription is now being used to subclassify pituitary adenomas according to lineage. Pit-1 is the transcription factor for somatotroph differentiation, PIT-1 with ER-α for lactotroph differentiation, and Pit-1 with GATA2 for thyrotroph differentiation. Corticotroph differentiation depends on Tpit and NeuroD1 and gonadotroph differentiation on SF-1 and GATA2 [(8)].

This study aims at using immunohistochemistry (IHC) for assessing transcription factors like pituitary transcription factor-1 (Pit-1), steroidogenic factor-1 (SF-1) and the hormonal markers of the pituitary gland along with the Ki-67 (MIB-1) proliferation index [(9)].

Additional IHC for CAM5.2, Pan-CK, Chromogranin A, Synaptophysin, TTF-1 and p53 was done, wherever required.



REVIEW OF LITERATURE



REVIEW OF LITERATURE

Anatomy:

The hypophysis or pituitary gland, is an ovoid and pea-sized gland, measuring approximately 12mm in transverse diameter and 8mm in anteroposterior diameter and weighs around 0.5 grams in adults. The gland rests upon the hypophyseal fossa of the sphenoid bone in the center of the middle cranial fossa and is surrounded by a small bony cavity called the sella turcica. The pituitary gland is surrounded by many important anatomic structures. Lateral to the pituitary are the cavernous sinuses containing the internal carotid arteries, venous plexuses and cranial nerves III through VI. Superiorly located are the optic chiasma and optic tracts, and inferiorly are the sphenoid bone and sphenoidal sinuses. It is flanked on both the sides by the wall of the cavernous sinus and antero-inferiorly by the posterior wall of the sphenoid sinus. This route through the sphenoid sinus is commonly used as the standard route for pituitary surgeries. Anterio-superiorly, the gland lies in close proximity to the optic chiasma [10,11].

This gland comprises the anterior lobe, the intermediate lobe and the posterior lobe.

The anterior pituitary, also known as adenohypophysis, constitutes the largest portion of the glandand has three components:

(1) The pars distalis,

(2) The pars intermedia and

(3) The pars tuberalis.

The intermediate lobe synthesizes and secretes melanocyte-stimulating hormones during fetal life. The intermediate lobe is almost rudimentary in adult life.

The posterior pituitary or the neurohypophysis is functionally connected to the hypothalamus by the median eminence via a small tube called the pituitary stalk or the infundibular stalk.

Embryology and Development:

The pituitary gland serves as an intermediary between the brain and the peripheral systems. Employing multiple feedback control mechanisms, the pituitary integrates incoming signals from the peripheral and central nervous systems and regulates production and secretion of critical regulatory hormones to target organs.

By the 12th week, pituitary development is essentially complete and the anterior gland starts showing immunoreactivity for different anterior pituitary hormones.

After birth, the gland grows rapidly and reaches a plateau at around 3 years of age. Another peak of rapid growth occurs between ages 10 years and 13 years of age.

The normal anterior pituitary gland is epithelial in origin whereas the posterior pituitary is derived from the neuroectoderm [12] .

Histology:

The gland is composed of relatively even sized clusters of cells arranged in acini. The acini are well delineated by reticulin stain. The anterior pituitary is comprised of different types of cells that secretes different types of hormones:

	Cell type	Hormones secreted	Percentage of cells
1	**Somatotrophs**	Growth hormone (GH)	30-40%
2	**Corticotrophs**	Adrenocorticotropin (ACTH)	20%
3	**Thyrotrophs**	Thyroid stimulating hormone (TSH)	3-5%
4	**Gonadotrophs**	Luteinizing hormone (LH) and Follicle stimulating hormone (FSH)	3-5%
5	**Lactotrophs**	Prolactin (PRL)	3-5%

Table 1: Types of cells in anterior pituitary

During organogenesis, the development of the gland occurs broadly through these following stages:

1.The initiation of pituitary organogenesis along with formation of the Rathke's pouch

2.Migration and proliferation of the Rathke's pouch

3.Lineage determination and cellular differentiation

A number of different pituitary specific transcription factors play significant roles in determination and growth of cell lineages. These are Pit-1, SF-1, Tpit, ER-alpha and GATA2.

Pit-1

The Pit-1 gene (POU domain, class 1, transcription factor 1 [POU1F1]) encodes a 33-kD, 291 amino acid transcriptional activator that is capable of DNA binding and transactivation, and it was initially isolated by its ability to bind to the responsive element of the GH gene promoter. Pit-1 is expressed exclusively in the pituitary gland. Although all pituitary cells contain Pit-1 mRNA, the protein is only found in these types of tumors: somatotroph, lactotroph, mammosomatotroph and thyrotroph adenomas [(13)].

SF-1

The nuclear receptor steroidogenic factor-l (SF-l) is a member of the steroid receptor superfamily and is necessary for the differentiation of pituitary gonadotrophs and is expressed in adenomas producing luteinizing hormone (LH) or follicle stimulating hormone (FSH) and has also been found to modulate the glycoprotein hormone α-subunit [(14)].

Tpit

Tpit (T-box transcription factor) along with NeuroD1 have been identified as restricted to proopiomelanocortinexpressing corticotrophs and melanotrophs (POMC)[(15)].

WHO Classification of Tumors of Pituitary, 4th Edition, 2017[(7)]

1.Pituitary adenomas

- Somatotroph adenoma
- Lactotroph adenoma
- Thyrotroph adenoma
- Corticotroph adenoma
- Gonadotroph adenoma
- Null-cell adenoma
- Plurihormonal and double adenomas

2. Pituitary carcinoma

3. Pituitary blastoma

4. Craniopharyngioma

- Adamantinomatous craniopharyngioma
- Papillary craniopharyngioma

5. Neuronal and paraneuronal tumors

- Gangliocytoma and mixed gangliocytoma-adenoma
- Neurocytoma
- Paraganglioma
- Neuroblastoma

6. Tumors of the posterior pituitary

- Pituicytoma
- Granular cell tumor of the sellar region
- Spindle cell oncocytoma
- Sellar Ependymoma

7. Mesenchymal and stromal tumors

- Meningioma
- Schwannoma
- Chordoma, NOS
  - Chondroid Chordoma
  - Dedifferentiated Chordoma
- Hemangiopericytoma/Solitary fibrous tumor
  - Grade 1 SFT/HPC
  - Grade 2 SFT/HPC
  - Grade 3 SFT/HPC

8. Hematolymphoid tumors

9. Germ cell tumors

- Germinoma
- Yolk sac tumor
- Embryonal carcinoma
- Choriocarcinoma
- Teratoma, NOS

- Mature teratoma
- Immature teratoma
- Teratoma with malignant transformation
- Mixed germ cell tumor

10. Secondary tumors

Prognostic features and grading[7]

Along with the morphological classification, other parameters of prognostic and diagnostic significanceare:

- Tumor size:
  - Microadenoma (<1cm)
  - Macroadenoma (1-4cm)
  - Giant adenoma (>4cm)
- Tumor's clinical presentation:
  - Clinically functioning
  - Clinically non-functioning or silent
- Invasiveness on MRI (Knosp grade) categorizes tumor proximity to the cavernous sinus
  - Non-invasive - Grade 1 and 2
  - Invasive -Grade 3 and 4
- Additional Immunohistochemistry
  - Chromogranin A
  - Low-molecular-weight cytokeratin : CK 7/8 (CAM 5.2), CK18
  - Proliferation markers: Ki-67, p53 (in limited cases)
  - Transcription factors and cofactors: Pit-1,SF-1,ER-α, Tpit, GATA2, NeuroD1
  - Predictive markers : SSTR2, SSTR5, MGMT, MSH6

Adenoma types	Morphological variants	Pituitary hormones	Transcription factors and co-factors
Somatotroph adenoma (SA)	Densely granulated SA	GH, α-subunit	Pit-1
	Sparsely granulated SA	GH	Pit-1
	Mammosomatotroph	GH+PRL (in same cells) ±α-subunit	Pit-1, ERα
	Mixed somatotroph-lactotroph	GH+PRL (in different cells) ±α-subunit	Pit-1, ERα
Lactotroph adenoma (LA)	Sparsely granulated	PRL	Pit-1, ERα
	Densely granulated	PRL	Pit-1, ERα
	Acidophil stem cell adenoma	PRL, GH (focal and variables)	Pit-1, ERα
Thyrotroph adenoma (TA)		β-TSH, α-subunit	Pit-1, GATA2
Corticotroph adenoma (CA)	Densely granulated	ACTH	Tpit
	Sparsely granulated	ACTH	Tpit
	Crookes cell adenoma	ACTH	Tpit
Gonadotroph adenoma (GA)		β-FSH, β-LH, α-subunit (various combinations)	SF-1, GATA2, ER-α
Null cell adenoma (NCA)		None	None
Plurihormonal adenoma	Histochemical combinations	Various combinations	Pit-1

Table 2: The 2017 WHO Morphological classification of pituitary adenomas along with transcription factors

For prognostication, The Ki-67 (MIB-1) Labeling Index (LI) is widely used.

Since Gerdes and colleagues described it for cell proliferation [16], the Ki-67 is used to assess the proliferative activity of tumor cells. MIB-1 is one of the most sensitive, commercially available Ki-67 equivalent antibodies. MIB-1 can react with an epitope of Ki-67 protein in formalin-fixed, paraffin-embedded sections. Ki-67 is expressed throughout the cell cycle except in the G_0 phase. As the cell cycle progresses from G_1 to M phase, Ki-67 protein accumulates to the maximum level and then falls rapidly after mitosis.

Currently, there is no other reliable molecular marker being used to predict the proliferative behaviour of pituitary adenoma. However, the examination of the Ki-67 labeling index is frequently used to gain insight into the growth rate and has been studied in relation to PA invasion. A study by Chacko et al, defined the biological significance of the MIB LI in 159 surgically excised PA and found that MIB1 correlated with PA invading the Cavernous Sinus [17]. The Ki-67 may also indicate possible residual PA/recurrent PA. The Ki-67 LI has been shown to be inversely correlated to Tumor volume doubling time (TVDT), where lower TVDT was associated with higher MIB-1. The impact that the Ki-67 marker has on pituitary adenoma invasiveness and recurrence remains controversial with conflicting reports in the literature. Nevertheless, this marker provides a general estimate of the proliferative potential of pituitary adenoma and is routinely included in pituitary adenoma pathology assessments [18].

Epidemiology and prevalence of Pituitary adenomas:

Pituitary adenomas are classically considered benign but the pathological variations and diverse clinical features often make targeted management difficult. High-resolution computed tomography (HRCT) and Magnetic resonance imaging (MRI) studies have revealed that approximately 20% of “normal” pituitary glands harbour an incidental lesion measuring 3 mm or more in diameter[19]. The majority of these tumors that are asymptomatic are clinically nonfunctioning tumors are now recognized to be of gonadotroph differentiation or prolactinomas that have not caused clinical symptoms recognized by the patient. In a meta-analysis of 10 studies (3 being radiological and 7 being post mortem studies) done by Ezzat et al, it has been seen that the prevalence is 16.7% in the general population[6]. The relative frequency of the various adenoma types in surgical series also varies with several factors like

geography and the therapeutic approach of the clinicians involved. Women usually present at a younger age and become symptomatic early and have a higher incidence of PRL-secreting adenomas and ACTH-secreting tumors, whereas men tend to present in middle or older age present with clinically nonfunctioning tumors. Pituitary adenomas are infrequent in childhood and only about 3.5–8.5% of pituitary adenomas are diagnosed before the age of 20 years.

Etiopathogenesis:

Although most of the pituitary adenomas are sporadic, a substantial number of these arise due to germline genetic abnormalities.

Some of the following syndromes are associated with increased predisposition to pituitary adenomas[(7)]:

- Multiple Endocrine Neoplasia Type 1
- Multiple Endocrine Neoplasia Type 4
- SDH-related familial paraganglioma and pheochromocytoma syndromes
- Carney complex
- McCune- Albright syndrome
- DICER 1 syndrome
- Neurofibromatosis (very rare)

Isolated pituitary diseases can be due to:

- Familial isolated pituitary adenoma
- X-linked acromegaly/gigantism syndrome

The findings of experimental studies suggest the tumorigenesis to be multifactorial and multiphasic, comprising of initiation and progression phases. It involves the loss of anti-oncogenic factors or the overexpression of oncogenic factors leading to clonal expansion of the cells of a single or multiple lineage.

Clinical features:

The signs and symptoms of pituitary adenoma include symptoms caused by the excess hormone produced by so-called functioning adenoma. In non-functioning adenomas, mass effects like bitemporal hemianopia, diplopia, facial numbness and headache are observed as

and when they increase in size to form macroadenomas. Large tumors can also press on the posterior part of the gland, thereby causing shortage of the hormone vasopressin.

The types of PA are[7]**:**

Somatotroph adenoma:

Lineage: Pit-1

Hormone secreted : Growth hormone (GH) [20].

Etiology and genetic profiling: Somatotroph adenomas are usually sporadic, but they can also arise due to genetic predisposition, as part of a syndromic disease or as an isolated pituitary disease. Somatic activating mutations in GNAS encoding G-protein alpha subunit (Gs-α), have been identified as many as 40-60% of somatotrophic adenomas.

Localization: Intrasellar but macroadenomas invade the cavernous sinus.

Clinical features: Increased levels of GH causes gigantism in children and acromegaly in adults. Other features are deepening of voice, prognathism, headache, vision change, joint pain, high blood sugar, kidney stones and heart diseases.

Diagnostic tests: The levels of GH and insulin-like growth factor-1 (IGF-1) in blood samples are increased.

Macroscopy: Soft and white to greyish-red. Macroadenomas often display invasion into adjacent structures. Tumors from patients with pituitary apoplexy contain haemorrhagic necrosis. Post-treatment adenomas may show fibrosis.

Microscopy: Disruption of the reticulin framework distinguishes adenoma from hyperplasia which shows an intact but expanded reticulin framework.

Based on their density of GH-containing secretory granules and their low-molecular-weight cytokeratin expression patterns, they are divided into two clinically relevant histological subtypes:

- DGSA: Densely granulated somatotroph adenomas and
- SGSA: Sparsely granulated somatotroph adenomas

DGSA are composed of tumor cells with eosinophilic cytoplasm,

Low-molecular-weight cytokeratin (CAM5.2 or CK18) immunohistochemistry shows characteristic perinuclear staining. Alpha subunit expression is seen.

Growth hormone-producing plurihormonal adenoma consists mainly of DGSAs,with variable thyrotroph and mammosomatotroph differentiation showing variable positivity for β-TSH, PRL, estrogen receptor, and low-molecular weight cytokeratin (perinuclear) is noted.

Prognosis and predictive features: Uncontrolled GH or IGF-1 secretion is associated with high morbidity and mortality. Surgery is the first-line treatment option for somatotroph adenomas.

SGSAs are less responsive to somatostatin antagonists and may require treatment with the GH-receptor antagonist pegvisomant. In such cases, somatostatin receptor detection by immunohistochemistry may be a useful predictor of treatment response.

Lactotroph adenoma:

Lineage: Pit-1

Hormone secreted : Prolactin (PRL)

Epidemiology:Lactotroph adenomas have a female predominance, with a peak incidence among patients aged 21 - 40 years. Although smaller in women, but in men they tend to present as invasive macroadenomas or giant adenomas (> 4 cm).

Etiology and genetic profiling: Estrogens play a role in the development of experimental lactotroph adenomas and during pregnancy. Few cases show genetic susceptibility for the syndromes already discussed above.

Localization: Lateral and posterior part of the anterior pituitary. Some tumors arise in ectopic locations such as the sphenoidal sinus and parapharyngeal region, clivus, middle nasal meatus, temporal bone, hypothalamus, and third ventricle as well as parasellar and suprasellar compartments.

Clinical features: Women in reproductive age group often present with menstrual irregularities, hypomenorrhoea and galactorrhea. In women who do not fall under reproductive age group, the diagnosis of prolactinomas is often missed. In men, increased prolactin levels cause gynaecomastia and erectile dysfunction. Features

common to both the sexes such as loss of libido, infertility and osteoporosis are often encountered.

Diagnostic tests: Pretreatment serum PRL levels.

Imaging: They appear hypointense when compared to the surrounding pituitary. Macroadenomas tend to have hemorrhagic features and show suprasellar extension, as well as downward invasion into sinuses and adjacent structures. Microcalcification is also noted.

Macroscopy: Microadenomas are expansile, compressive lesions, often widely invasive, have soft consistency and reddish-tan appearance with a pseudo capsule. Some tumors are firm and greyish-white, containing abundant amyloid stroma. Fibrosis, cystic changes, and calcification may be present in macroadenomas and previously treated tumors.

Microscopy: Classified into three clinically relevant histological subtypes:

- SGLA: Sparsely granulated lactotroph adenoma,
- DGLA: Densely granulated lactotroph adenoma and
- ASCA: Acidophil stem cell adenoma

- SGLA is composed of chromophobic tumor cells that stain negative for PAS. They display a large variety of growth patterns. Psammoma bodies, interstitial fibrosis or amyloid of endocrine type that stains may be present.
- DGLA shows eosinophilic to acidophilic cytoplasm and displays strong and diffuse cytoplasmic expression of PRL hormone.
- ASCA shows oncocytic features.

All the lactotroph adenomas show cytoplasmic PRL expression and express PIT-1 and ER-α. Additionally, the ASCA subtype shows variable growth hormone expression and clinically also may show features of acromegaly.

Prognosis and predictive features: Pharmacotherapy with dopamine agonists like cabergoline is the preferred treatment for achieving normoprolactinemic states. Patients who have invasive macroadenomas with resistance to pharmacotherapy or who are intolerant to medications may require transsphenoidal surgery. The ASCA subtype is of clinical

significance because they tend to be more aggressive. Radiotherapy and chemotherapy with temozolomide is used in resistant and recurrent tumors.

Thyrotroph adenoma

Lineage: Pit-1

Hormone secreted : Thyroid stimulating hormone (TSH)

Epidemiology: Rare , < 2% of all pituitary adenomas

Etiology and genetic profiling: The etiology of most tumors is largely sporadic.

Localization: Sella Turcica. Ectopic thyrotroph adenoma can be seen in the nasopharynx.

Clinical features: Thyrotroph adenomas cause symptoms of hyperthyroidism characterized by arrhythmias, tremor, weight loss, change in appetite, changes in bowel movements, sweating, anxiety and insomnia. There may be an enlarged lump in front of the neck.

Diagnostic tests: Although these tumors are rare causes of hypothyroidism, TSH immunometric assays have to be correlated with imaging modalities as it can stay unrecognized and can cause grave consequences.

Imaging: In MRI studies, these tumors are macroadenomas and are frequently invasive.

Macroscopy: The average tumor size of clinically nonfunctioning (silent) thyrotroph adenoma is significantly larger than that of clinically functioning thyrotroph adenomas.

Microscopy: The tumors are chromophobic polygonal or short-spindled cells with pleomorphic nuclei, usually arranged in a diffuse pattern and exhibiting a globoid or whorl-like appearance, with intertwined cytoplasmic processes. Stromal fibrosis and calcification are often noted.

The tumor cells are frequently positive for both TSH-beta and alpha subunit.

Thyrotroph adenomas frequently co-express growth hormone and prolactin without clinical manifestations.

Prognosis and predictive features: With trans-sphenoidal surgery, total removal with endocrine remission achieved is 84% cases.

Corticotroph adenoma

Lineage: Tpit

Hormone secreted :ACTH and other proopiomelanocortin derived peptides (POMC).

Epidemiology: Cushing syndrome occurring secondary to corticotroph adenoma is referred to as Cushing's disease seen in approximately 15% of all pituitary adenomas. The peak incidence is in patients aged 30-50 years along with male predominance in prepubertal patients, whereas a female preponderance is seen in adults.

Etiology and genetic profiling: Recent studies have highlighted the role of the EGFR signaling pathway in the pathogenesis of corticotroph adenomas.

Localization: Mostly in pituitary gland. However, ectopic adenoma cells are seen in the cavernous sinus, orbit, clivus and suprasellar region.

Clinical features: Excess of adrenocorticotropic hormones predisposes to Cushing's disease. Decreased libido and changes in menstrual cycle are noted. Weakness of bones leading to osteoporosis and fractures are often encountered.

Diagnostic tests: Serum cortisol levels.

Imaging: In MRI studies, most Cushing's diseases present as solitary microadenomas.

Macroscopy: Soft and white to grayish-red.

Microscopy: Classified into three subtypes:

- DGCA: Densely granulated corticotroph adenoma,
- SGCA: Sparsely granulated corticotroph adenoma and
- Crooke cell adenoma

Densely granulated corticotroph adenomas are composed of basophilic PAS-positive cells that are diffusely and strongly positive for ACTH.

Sparsely granulated corticotroph adenomas are composed of faintly basophilic or chromophobe PAS-positive cells with weak or patchy positivity for ACTH, consistent with the scant, small secretory granules seen ultrastructurally.

Crooke cell adenomas are composed of tumor cells with Crooke hyaline change. Ring-like cytokeratin expression is typical of these neoplasms.

The corticotroph adenomas stain with ACTH.

Prognosis and predictive features: Surgery is the treatment of choice and is often curative for microadenomas but less so for macroadenomas. Silent corticotroph adenomas and Crooke cell adenomas show a clinically more aggressive behavior. Few respond to temozolomide.

Gonadotroph adenoma

Lineage: SF-1

Hormone secreted : β-FSH, β-LH and/or α-SU

Epidemiology: In pathological studies of surgically removed pituitary adenomas, 25-29% were diagnosed as clinically non-functioning adenomas and 43-64% were classified as gonadotroph adenomas. Gonadotroph adenomas usually occur in middle-aged and older people, with a slight male preponderance, and are rare among individuals aged < 25 years.

Etiology and genetic profiling: Generally arise spontaneously and may be secondary to long standing primary hypogonadism.

Localization: Usually occur as macroadenomas with suprasellar and parasellar extensions.

Clinical features: Manifestations are irregular menstruation, low testosterone levels, infertility and loss of libido. Many gonadotroph adenomas often are silent and are detected as and when they increase in size to cause pressure symptoms.

Imaging: In MRI studies, it appears as macroadenoma.

Macroscopy: Gonadotroph adenomas are well-vascularized, soft, tan to dark-brown tumors. They rarely appear as cystic mass, often containing areas of necrosis or hemorrhage.

Microscopy: The tumor cells are chromophobic and give a negative to weak PAS reaction. Variable oncocytic change may be seen. A distinctive perivascular papillary

pseudorosette arrangement is a consistent and prominent histological feature of gonadotroph adenomas but diffuse and sinusoidal arrangements are also seen.

Diffuse and strong immunopositivity for FSH-beta, LH-beta, or alpha subunit is noted, FSH-beta type being most common and LH-beta least common type. Instead, most adenomas are immunopositive for one or more gonadotropin subunits.

Prognosis and predictive features: Tumor invasion, particularly in the cavernous sinus is the major risk factor.

High-risk adenoma:

The WHO 2017 classification collectively refers to sparsely granulated somatotroph adenoma, lactotroph adenoma in men, Crooke's cell adenoma, silent corticotroph adenoma, and plurihormonal POU class 1 homeobox 1-positive adenoma as 'invasive adenoma' or 'high-risk adenoma'. This type of adenoma grows rapidly, tends to recur or progress and is resistant to surgery and radiotherapy.

Silent adenomas:

Silent adenomas are clinically asymptomatic, have low levels of serum hormones and are immunohistochemically positive for certain hormones.

Null cell adenoma:

Null cell adenoma was redefined as an adenoma composed of anterior pituitary cells, and on IHC, pituitary hormone detection and transcription factor detection showed no evidence of cell-specific differentiation. They usually present with features of mass effect and comprise of 5-30% of all PAs.

Invasive adenoma:

Although the WHO recommendations do not currently include invasive tumors in the classification, the 2017 classification highlights that invasive adenoma can be considered as a significant feature for detecting clinically refractory adenomas and assessing prognosis. Preoperative MRI and intraoperative evidence of tumor dissemination to the dura mater, bone, or nasal mucosa can be used to detect invasive growth.

In 1993, Kenichi Inada et al studied the role of Pit-1 in functional differentiation of the anterior pituitary by doing immunohistochemical studies in 86 pituitary adenomas. His cohort consisted of 51 GH-producing adenomas, 18 nonfunctioning adenomas, 11 PRL-omas, and 6 TSH-omas. Pit-1 was found to be positive in 44.2% of human pituitary adenomas that they studied. 100% of TSH-omas, 52.9% of GH-omas and 11.1% of non-functioning adenomas showed nuclear positivity for Pit-1 [(20)].

In 1996, JR Bharadwaj et al studied 20 cases of pituitary adenoma by light microscopy, immunohistochemistry and Electron Microscopy and divided the patients into 2 groups: Group I comprising of 12 patients having no endocrinal dysfunction and Group II comprising of 8 patients with endocrinal dysfunction. They found that out of the 12 patients in group I, 6 patients showed hormonal production on immunohistochemistry. Moreover, 8 of the 12 patients were plurihormonal wherein, Growth hormone and Prolactin were the commonest combination. They further concluded that sometimes the serum hormonal levels was not always elevated and was not a correct representation of clinical picture, thus , suggesting the use of IHC to be necessary [(21)].

In 1996, S L Asa studied the expression of transcription factor SF-1 in 32 pituitary adenomas. They applied both RT-PCR analysis and immunohistochemistry and found that 8 gonadotroph adenomas had a strong signal for SF-1, thus implicating that SF-1 is cell cycle specific transcription factor that regulated gonadotropic differentiation in pituitary. Additionally, SF-1 mRNA bands were also noted in 2 of the 3 corticotroph adenomas and 2 somatotroph adenomas and one lactotroph adenoma also showed faint mRNA bands as well. But immunocytochemical studies only shows SF-1 positivity in the 8 gonadotroph adenomas and only showed focal positivity in non-tumorous adenophysis [(14)].

In 2004, Pizzaro et al did a study on Ki-67 labelling index on 159 pituitary adenomas. Their mean Ki-67 index was 1.22 and the range was 0.16 to 15.48% . The Ki-67 index did not show any statistically significant difference between gender, age, secretory or non-secretory tumors. They found invasive tumors to have a higher value of Ki-67 labelling index than non-invasive tumors [(22)].

In 2016, William C. McDonald et al studied 136 pituitary adenomas and the expression of steroidogenic SF-1, Pit-1, anterior pituitary hormones, cytokeratin CAM5.2, and α-SU of human chorionic gonadotropin in a 2-step algorithm. They compared 6 hormonal IHC markers of anterior pituitary to an alternate approach comprising of a screening step in which

IHC for transcription factors like Pit-1, SF-1 along with adrenocorticotropic hormone was used followed by an additional step for Pit-1 family members and plurihormonal tumors using IHC for PRL, GH, β-TSH, and CAM5.2. Their algorithm uses approximately one third fewer IHC stains and detects gonadotroph adenomas with greater sensitivity [23].

In 2017, Sen A et al did a prospective study on 34 cases of pituitary tumors. A cohort of 32 cases was taken and IHC markers for GH, PRL and ACTH along with Ki-67 LI were applied. They found GH-omas to be the most common (36%) followed by PRL-omas (19%). The mixed PRL+GH-omas were found to be positive in 16% cases and ACTH-omas were found in 10% cases. They also found serum levels and IHC marker positivity of the different hormones to be corroborative in only 32% of cases. Since they did not apply other hormonal or immunohistological markers, they refrained from calling the remaining 19% cases as null cell adenoma. They found Ki-67 LI to be <3% in 87% of pituitary adenomas [24].

In 2017, BM Kwinta et al analyzed 72 cases of pituitary adenomas and found 22 monohormonal adenomas (30.56%), 21 plurihormonal adenomas (29.17%), 21 immunonegative adenomas (29.17%) and 8 unreliable cases (11.11%). The immunopositivity for particular hormones were PRL and GH (25% each), α-SU (22.22%), ACTH (13.89%), LH and FSH (12.5% each), and TSH (5.56%) [25].

In 2018, Tiwari S et al comprehensively studied the newer basis of WHO classification, 4th edition, and discussed the anatomy, development, histology and molecular pathology and the role of transcription factors like SF-1, Pit-1 and Tpit in the development of pituitary adenomas [26].

In 2019, Petry et al did a retrospective study on the Ki67 proliferative index. The aggressive course of a number of pituitary adenomas requires the investigation of potential predictors. They included 52 patients with pituitary adenoma and divided patients according to Ki67 expression into high (≥3%) vs. low (<3%) levels of Ki67. The Ki67 index ranged from 0 to 30%; In 23 cases, Ki67 was ≥3%. The patient with the highest Ki67 index (30%) developed pituitary carcinoma [9].

In 2019, Aydin S et al found 27 out of 665 cases of pituitary adenomas to be plurihormonal adenomas. 18 were found to be plurihormonal adenomas with unusual immunohistochemical combinations and 9 patients were diagnosed with PIT-1+ PPAs. 24 patients (88.8%) had macroadenomas, including 6 giant adenomas (≥4 cm) (22.2%). Cavernous sinus invasion was found in 12 patients (44.4%). Pathologic examinations showed high aggressivity in nearly

half of the patients. Most patients with unusual immuhistochemical combinations (77.8%) had features of nonfunctioning pituitary adenomas, and only 4 had features of hormone-secreting pituitary adenomas [27].

In 2020, Liu et al studied 250 cases of pituitary adenomas and classified them according to the 2017 WHO classification. Of the 250 cases, 45 cases were diagnosed as somatotroph adenomas, 26 cases as lactotroph adenomas, 1 case as thyrotroph adenoma, 61 cases as corticotroph adenomas, 93 cases as gonadotropin adenomas, 15 cases as null cell adenomas and 9 cases as plurihormonal adenomas. There were 5 types of high-risk pituitary adenoma identified: 17 cases of sparsely granulated somatotroph adenoma, 11 cases of lactotroph adenoma in men, 3 cases of plurihormonal PIT-1 positive adenoma and 42 cases of silent corticotroph adenoma. They did not find any Crooke's cell adenoma. High risk and recurrent adenomas were found to have a higher rate of Ki-67 proliferative index [28].

In 2020, Tamanini et al digitally assessed the percentage of Pit-1, SF-1 and Tpit positive cells in 10 patients with acromegaly, 10 patients with Cushing's disease and 10 NFPA patients. He found frequency of Pit-1 to be more in patients in acromegaly and that of Tpit to be more in patients with Cushing's disease. SF-1 was unable to differentiate NFPA from other two groups [29].

In 2021, Pernik et al did a meta-analysis and found 1057 cases of non functioning pituitary adenoma (NFPA) in the 19 articles they reviewed. They found that macroadenomas are more likely to undergo recurrence and apoplexy as compared to microadenomas. So the identification of NFPA are of immense importance for prognostication as they warrant close follow up post operatively [30].

AIM & OBJECTIVES

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AIM

- To study the expression of Pit-1 and SF-1, Ki-67 labelling index and other IHC markers namely PRL, GH, ACTH, β-LH, β-FSH and β-TSH in pituitary adenomas.

OBJECTIVES

- To assess the expression of Pit-1, SF-1, Ki-67, and the hormonal IHC markers of anterior pituitary namely PRL, GH, ACTH, β-LH, β-FSH and β-TSH.
- To find out the number of cases of the specific types of pituitary adenomas and then to classify them according to lineage and then subtyping them further by applying the specific hormonal markers belonging to the specific lineage.

MATERIAL & METHODS

MATERIAL AND METHODS

STUDY DESIGN:

Ambispective (Prospective and retrospective) hospital based observational study.

STUDY SETTING:

Department of Pathology & Lab Medicine, Department of Endocrinology and Metabolism and Department of Neurosurgery, All India Institute of Medical Sciences, Jodhpur.

DATA collection:

This study included biopsy specimens of diagnosed cases of Pituitary Adenoma. These were received in the Department of Pathology & Lab Medicine at AIIMS Jodhpur for histopathological examination from January 2016 to December 2021.

Patient's detailed medical records and personal information such as sex, age at diagnosis, clinical manifestations, co-morbidities, relevant medical events with particular importance to endocrinal manifestations, and details about surgical procedure, and CSF findings, if any, were collected.

The institutional ethics committee approved the study on 01/01/2020, bearing Certificate No: AIIMS/IEC/2019-20/966.

INCLUSION CRITERIA:

All diagnosed cases of pituitary adenomas

EXCLUSION CRITERIA:

- Any other central nervous system neoplasm excluding pituitary adenomas.
- Inadequate sample in formalin fixed paraffin embedded blocks.

SAMPLE PROCESSING, STAINING AND IMMUNOHISTOCHEMISTRY

After approval from the Institutional ethics committee, the study was started. Informed consent was obtained from the patients. The type of surgical procedure done, the extent of

disease and clinical parameters, radiological and biochemical hormonal levels were recorded. Patient details were received from the Department of Neurosurgery at AIIMS, Jodhpur with the histopathology requisition form. Biopsy specimens were fixed and measured in three dimensions. The entire specimen was submitted in an appropriate number of cassettes.

Thin sections (3-5µm) were stained with routine Hematoxylin and Eosin. On light microscopy, all relevant findings were recorded. The appropriate representative block was subjected to IHC and assessed.

STEPS OF BLOCK PREPARATION AND SECTION CUTTING

After the representative sections were taken from the specimen, tissue was processed in an automated tissue processor as follows:

1. **Dehydration** was carried out by passing the sections through a series of ascending grades of ethyl alcohol, from 50%, 70%, 95% to absolute alcohol.

2. **Clearing** was done by passing the tissue through two changes of xylene.

3. **Impregnation** was done in molten paraffin wax with a melting point of 54 – 62˚C.

4. **Embedding** was done using the Embedding station (Leica EG 1150 H).

5. **Microtomy**: Microtome (Leica-RM2255) was used and thin ribbons (3-5 µm) were cut and floated in warm water at 56˚C. These sections were then collected on frosted glass slides and kept for drying.

STAINING OF SECTIONS

- **For Hematoxylin and Eosin Stain[31]:**

1. Deparaffinization – The glass slides containing the tissue sections were kept over a hot plate at 60 °C for 10 minutes, followed by dipping twice in xylene (Xylene I & Xylene II), 10 minutes each.

2.Hydration – The slides were graded through the alcohol (100%, 95%, 70%, 50%) to water, 10 minutes each.

3. **Hematoxylin** – The sections were kept in hematoxylin for 5 minutes.

4. **Washing** – The sections were washed well in water for 2 minutes.

5. **Differentiation** – Done in 1% acid alcohol (1% HCl in 70% alcohol) for 10 seconds.

6. **Washing** – Done under running tap water (usually for 15- 20 minutes) until the sections blue.

7. **Eosin** – The slides were stained in 1% Eosin Y for 10 seconds.

8. **Washing** – Done in running tap water for 2 minutes.

9. **Dehydration** – Done through graded alcohol (50%, 70%, 95%, 100%), 10 minutes each.

10. **Clearing** –Through xylene (Xylene II & Xylene I), 2 minutes each.

11. **Mounting** – The sections were mounted in DPX with a coverslip.

- **HISTOCHEMICAL STAINS**

Reticulin stain was done to for assessing the adenomatous area/ foci, which predominantly showed disruption of acinar pattern and PAS (Periodic acid-Schiff) stain was also done.

- **IMMUNOHISTOCHEMISTRY**

Primary antibodies used:

Primary antibody	Company	Catalogue Number	Dilution	Quantity in Vial
Pit-1	Novus Biologicals	NBP1-92273	1:500	25µl
SF-1 Polyclonal Antibody	Invitrogen, Thermo Fisher Scientific	Catalog:PA536103 (Lot # SI2447841)	1:150	100 µl
Ki-67 (MIB-1)	Thermo Fisher Scientific	RM-9106-R7	Ready to use	7ml
GH	Thermo Fisher Scientific	MS-1328-R7	Ready to use	7ml
PRL	Thermo Fisher Scientific	MS-1362-R7 (clone PRL02)	Ready to use	7ml
β-TSH	Thermo Fisher Scientific	MS-1453-R7	Ready to use	7ml
β-FSH	Thermo Fisher Scientific	MS-1449-R7	Ready to use	7ml
β-LH	Thermo Fisher Scientific	MS-1448-R7	Ready to use	7ml
ACTH	Thermo Fisher Scientific	MS-452-R7	Ready to use	7ml

Table 3: Primary antibodies

Secondary Antibody: Bond Polymer Refine Detection, Leica

- Peroxide block, 3-4%(v/v)

- Post Primary, Rabbit anti mouse IgG in 10% (v/v) animal serum in tris-buffered saline

- Polymer, Anti-rabbit Poly-HRP-IgG containing 10% (v/v) animal serum in tris-buffered saline

- DAB Part 1, in stabilizer solution

- DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
- DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution

- Hematoxylin, 0.1%

STEPS OF IHC STAINING:

A. Preparation of Buffer–Two types of buffers were used.

1. Wash Buffer
2. Antigen Retrieval Buffer (ARB)

Wash buffer preparation: 6 gm powdered TRIS buffer salt was dissolved into 1 liter of distilled water and pH was set at 7.4.

ARB preparation:

1. TRIS Buffer: 6.05 gm TRIS salt and 0.744 gm EDTA salt dissolved in 1 liter of distilled water, and the pH set at 9.0.

2. Citrate Buffer: 29.40 grams of sodium citrate in 1 liter of distilled water, and the pH set at 6.0. A citrate buffer was used for the hormonal markers.

NOTE:

- To increase the pH, NaOH solution was added drop by drop and pH was titrated.
- To decrease the pH, HCl was added drop by drop and pH was titrated.

B. Preparation of Poly-L-Lysine Solution (PLL Solution):

1 ml of PLL was diluted with 9 ml of distilled water (1 in 10 dilutions).

C. Slide Coating Procedure:

Step 1: Diluted PLL solution was taken in a clean container/Coplin jar.

Step 2: Both sides of the glass slides were cleaned with tissue paper.

Step 3: The clean slides were immersed in PLL solution for 5 minutes.

Step 4: After 5 minutes, the coated slides were removed and kept overnight for air drying. Tissue sections of 4 μ thickness were obtained on the PLL coated slides.

IHC STAINING PROCEDURE

All steps were manually done.

Step 1, Deparaffinization – The slides were kept in the xylene I (10 minutes), followed by Xylene II (10 minutes).

Step 2, Rehydration – The slides were kept in 100%, 70%, and 50% alcohol for 5 minutes, each followed by running tap water for 5 minutes.

Step 3, Antigen retrieval – by pressure cooker method. 200 ml of clean tap water was taken in the empty pressure cooker and heated up to the steam formation. The slides were placed in a rack. 300 ml of the ARB was put in the container and the rack with slides placed inside the container. The container containing the rack with slides was placed in the pressure cooker, and the lid was closed. After two whistles, the pressure was released by lifting the air vent and cooling it until it reached room temperature.

Step 4, Wash – slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 5, Peroxide blocking – Blocking reagent was added to the sections and incubated for 10 minutes in the humidity chamber at room temperature. This step prevents unwanted, nonspecific background staining. The peroxide was decanted and not washed with buffer.

Step 6, Primary antibody –SF-1, Pit-1, KI-67, PRL, GH, β-TSH, β-LH, β-FSH, and ACTH were manually added to the sections and incubated in Humidity chamber for one hour. After that, slides were washed in wash buffer (pH 7.4) thrice at a 1-minute interval.

Step 7, Post-primary was added over the sections and incubated for 10 minutes in the humidity chamber at room temperature.

Step 8, Wash – The slides were washed in wash buffer (pH 7.4) thrice at a 1-minute interval.

Step 9, HRP label – The HRP was added and incubated for 30 minutes in the humidity chamber at room temperature.

Step 10, Wash – The slides were washed in wash buffer (pH 7.4) thrice at a 1-minute interval.

Step 11, DAB – The DAB chromogen was applied to the sections and incubated in the humidity chamber for 10 minutes, avoiding light exposure as much as possible.

Step 12, Wash – The sections were washed in distilled water twice at a 1-minute interval.

Step 13, Counterstain – Slides were counterstained using Hematoxylin for 2-3minutes.

Step 14, Wash – The slides were washed in running tap water for 5 minutes.

Step 15, Dehydration – was done in graded alcohol (50%, 70%, 95%, 100%), 1 minute each.

Step 16, Mounting – Slides were air-dried, then mounted with DPX, and examined under microscope.

Interpretation of IHC:

IHC was performed using commercially available antibodies for Pit-1, SF-1, KI-67 (MIB-1), GH, PRL, ACTH, β-LH, β-FSH, and β-TSH. With each batch, positive controls from previously positive and standardized tissue were also run.

We reviewed all the slides along with their corresponding H&E sections and reticulin stained slides and ruled out all parts of specimens with normal pituitary tissue, fibrosis, hemorrhage, etc. Staining observed in entrapped normal cells was omitted from interpretation.

Additional IHC for CAM 5.2, Pan-CK, Chromogranin A, Synaptophysin, TTF-1 and p53 was also performed so as to aid in the refined classification of cases.

Hormonal Markers:

Diffuse cytoplasmic staining was observed in the hormonal markers (GH, PRL, ACTH, β-LH, β-FSH, and β-TSH). Staining in more than 10% of tumor cells was considered positive.

Transcription factors:

For the transcription factors (Pit-1 and SF-1) staining in more than 5% of cells was considered for further scoring.

Stains were assessed using the Allred scoring method, a semiquantitative method extensively employed in hormone receptor analysis of breast cancer[32].

Positive Cells (%)	Proportion Score	Intensity	Intensity Score
0	0	**None**	0
<1	1	**Weak**	1
1-10	2	**Intermediate**	2
11–33	3	**Strong**	3
34–66	4		
>/=67	5		

Table 4: Allred Score for hormone receptor analysis

The Allred score combines the percentage of positive cells and the intensity of the reaction product. The 2 scores are added together for a final score with 8 possible values.

- Scores of 0 and 2 are considered negative.
- Scores of 3-8 are considered positive.

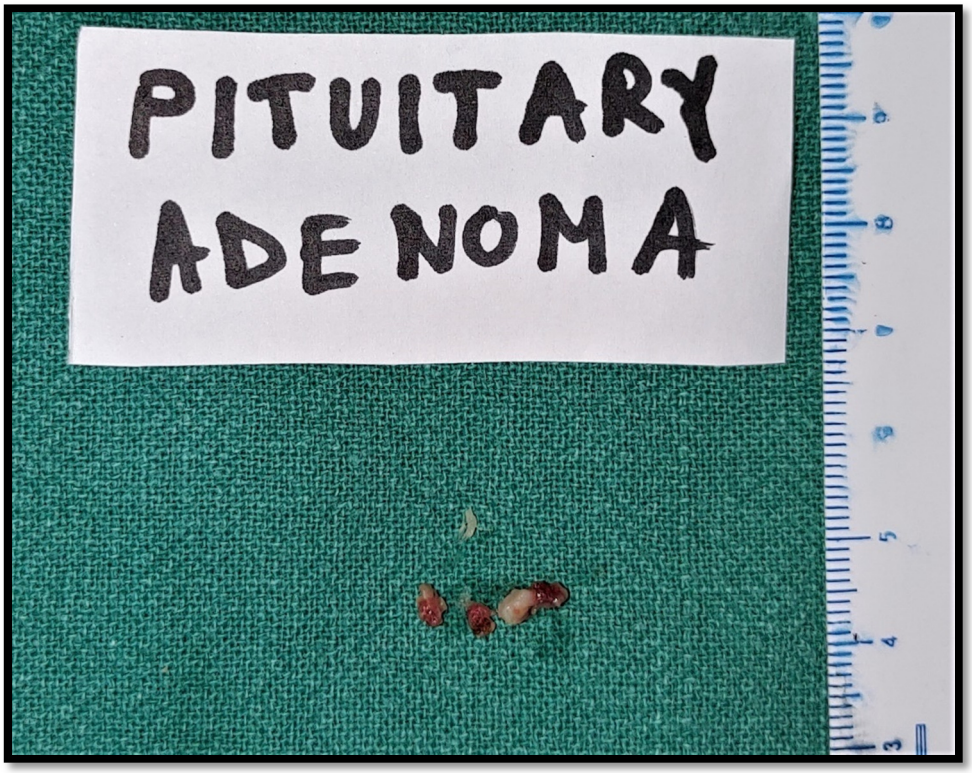
Interpretation of Ki-67 labelling Index

The Ki-67 LI was defined as the percentage of tumor nuclei taking up the stain to all the tumor nuclei. Cells showing diffuse or granular brown nuclear stain were taken as Ki-67 positive. Necrosis, normal brain tissue, zones of infiltration, endothelial and inflammatory cells were omitted from the evaluation. A whole slide mean value at a hot spot was estimated for each tumor. To estimate the whole slide mean values, the tumor slides were viewed using the scanning objective (40× magnification) with subsequent evaluation at higher magnification of selected foci of viable tumor areas (400× magnification). After selection of the hot spot, 500 cells were counted. Cells showing nuclear positivity irrespective of intensity were considered positive. The mean value of Ki-67 was tabulated. The reported value was expressed in percentage [(16)].

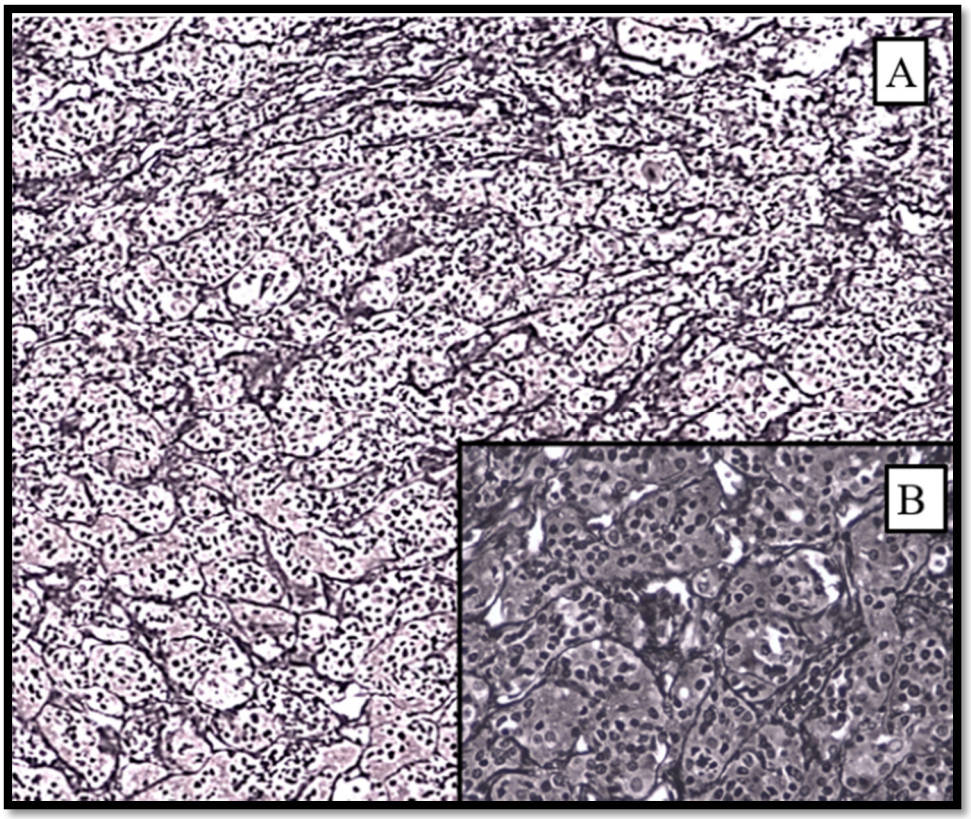
Statistical Analysis

Based on a review of records of the Department of Pathology& Lab Medicine, all the cases from January 2016 till December 2021 were included.

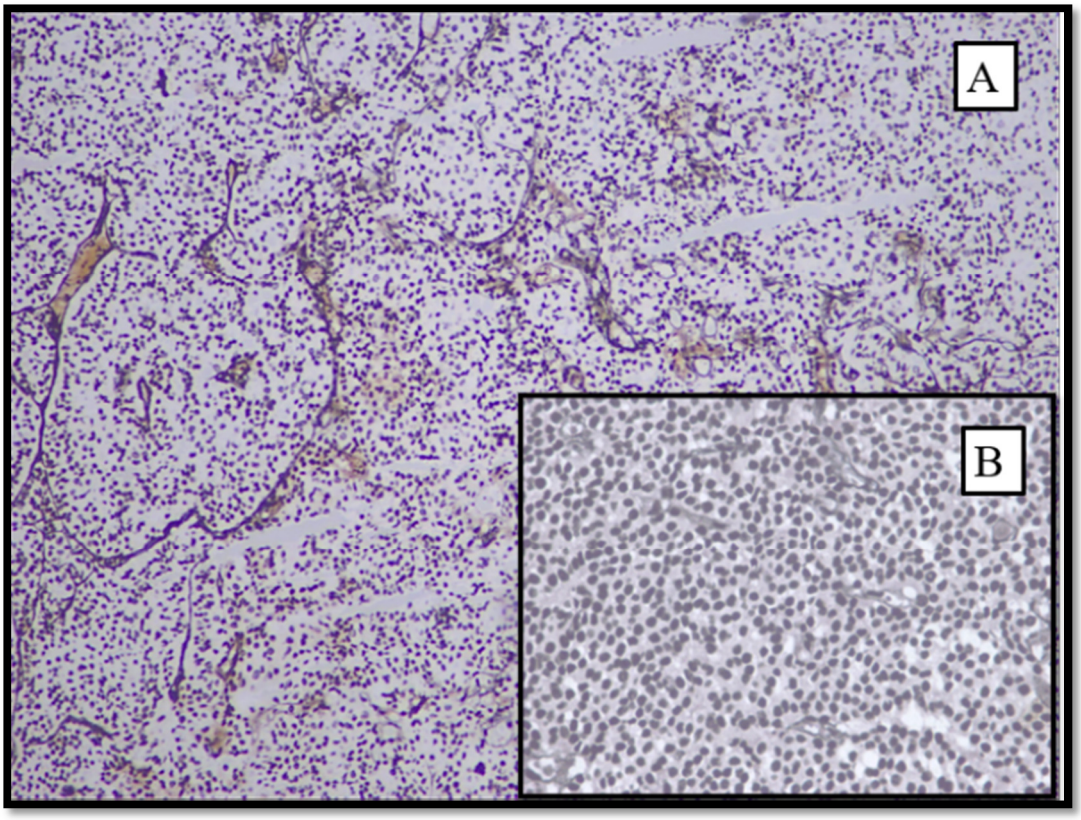
Data was entered in excel sheet and analysed by IBM-SPSS (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.) software. To check the association of categorical variables, Spearman's correlation coefficient (ρ) was calculated.



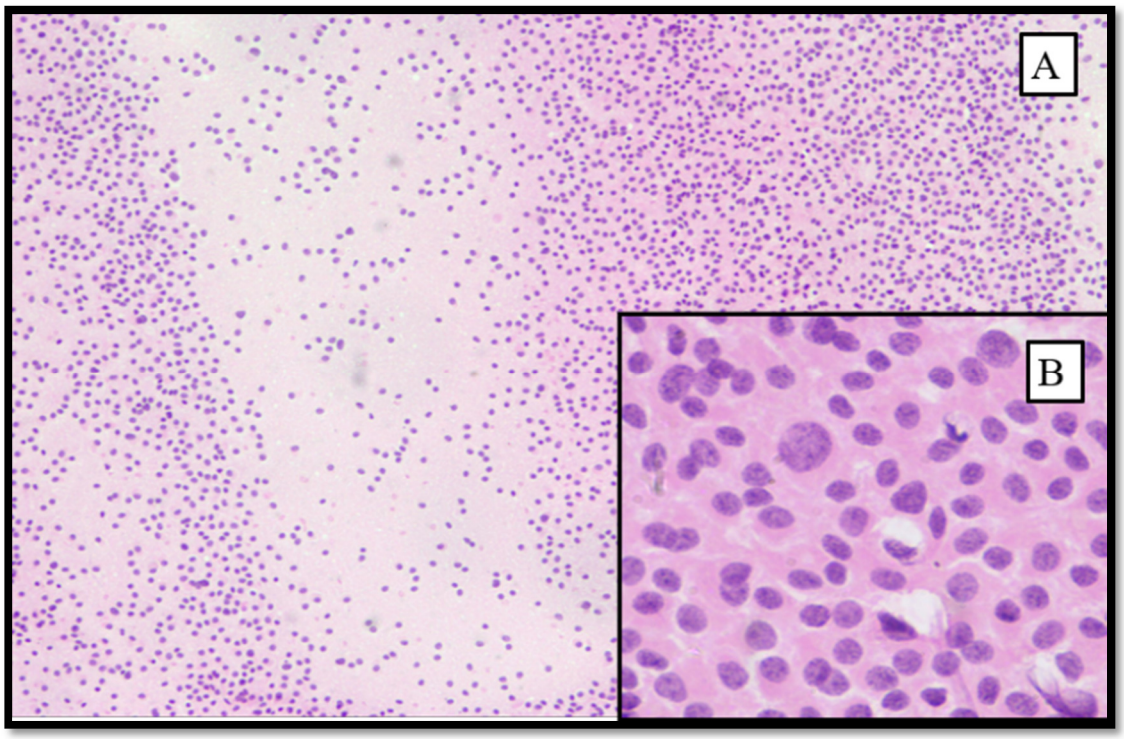
Color plate 1: Gross picture of pituitary adenoma specimen



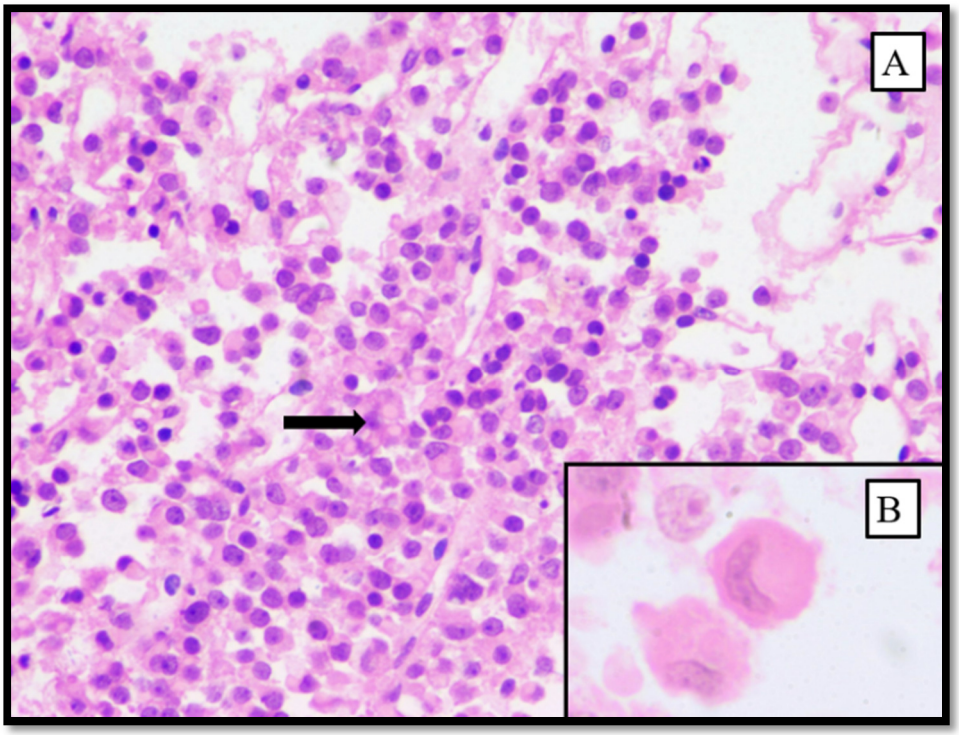
Color plate 2: Acinar arrangement of normal pituitary gland (A) Reticulin stain, 100x, (B) Inset, 400x



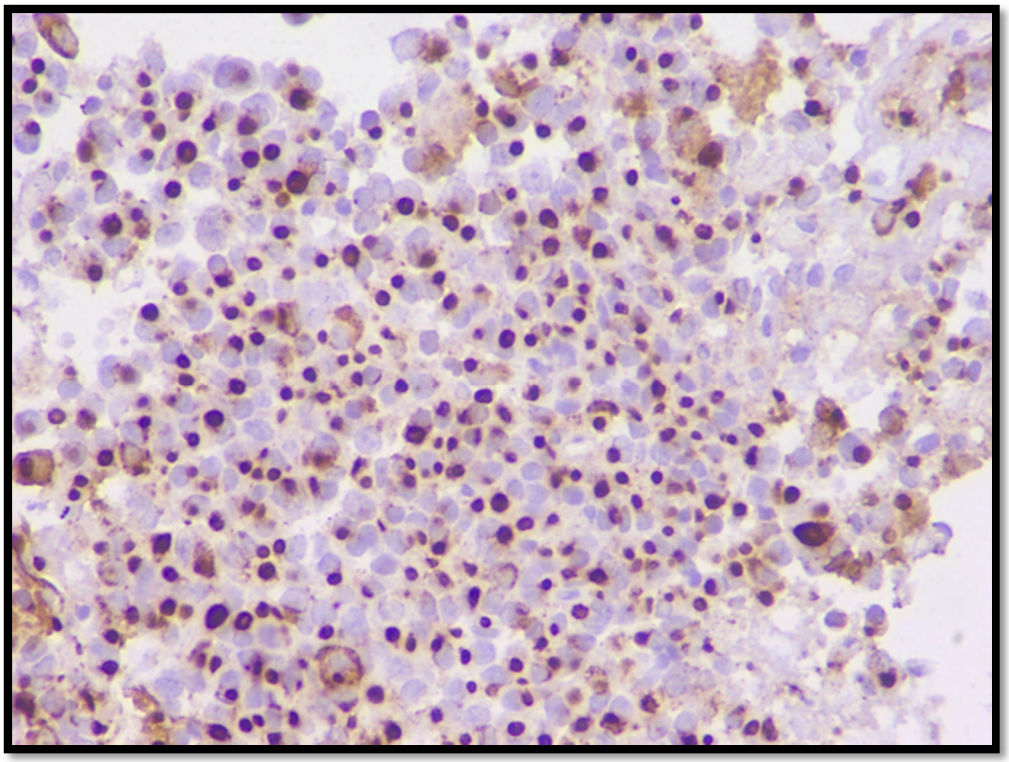
Color plate 3: Disrupted acinar arrangement of pituitary adenoma (A) Reticulin stain, 100x, (B) Inset, 400x



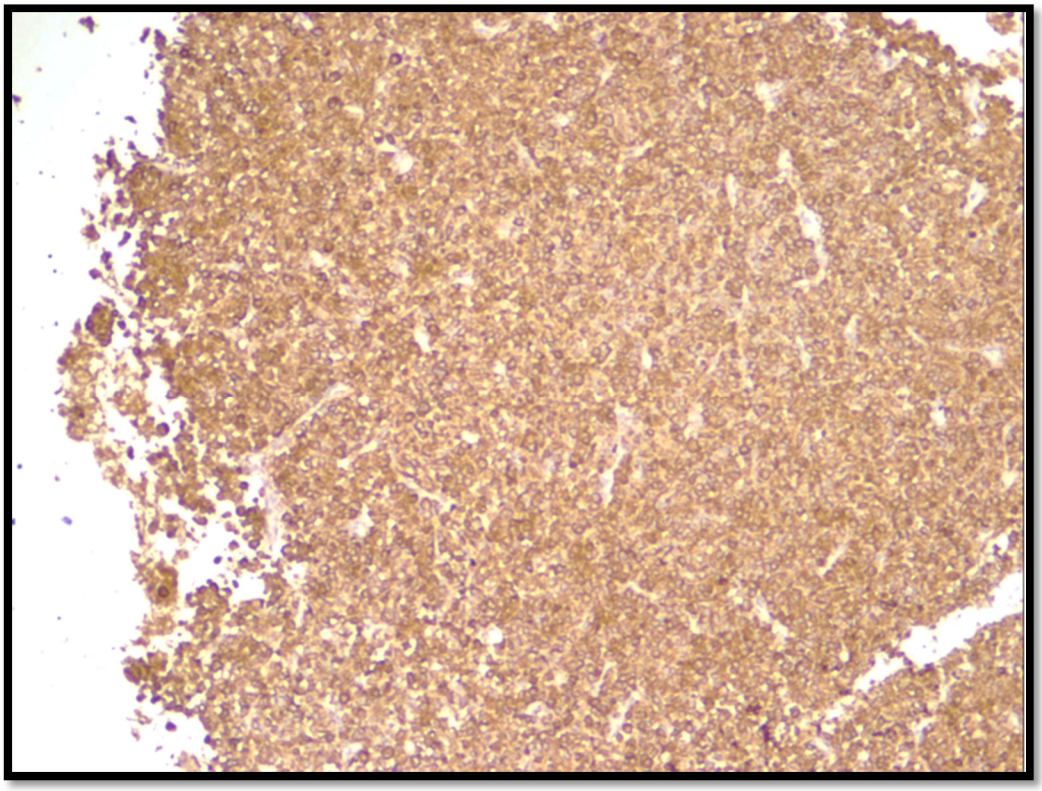
Color plate 4: Frozen section showing sporadic nuclear atypia (A) H & E, 100x, (B) Inset, 400x



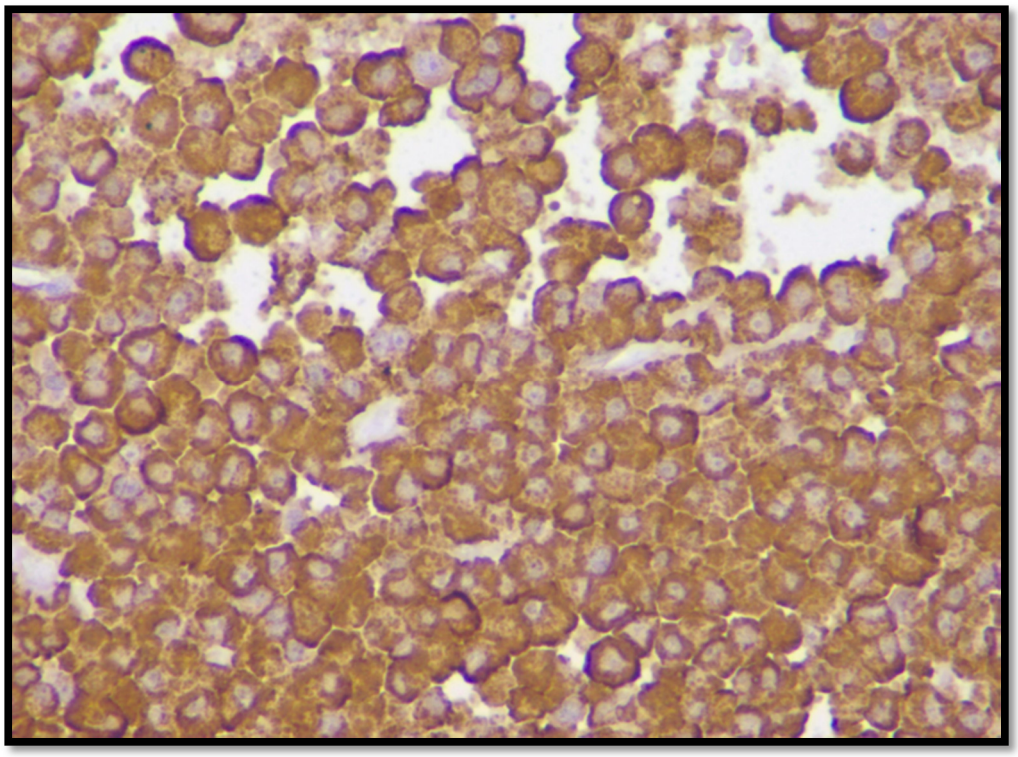
Color plate 5: Fibrous bodies in Somatotroph adenoma (A) H & E, 400x (B) Inset, oil immersion, 1000x



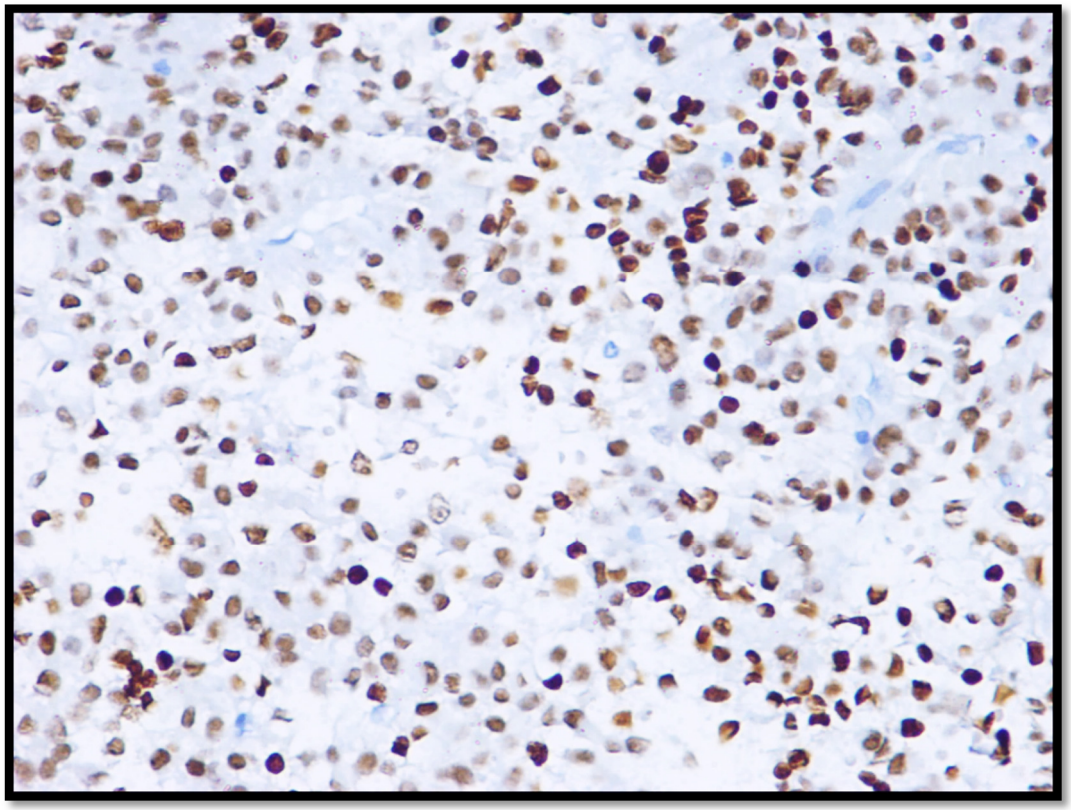
Color plate 6: Cytokeratin (CK) IHC showing perinuclear dot like positivity in Somatotroph adenoma, 400x



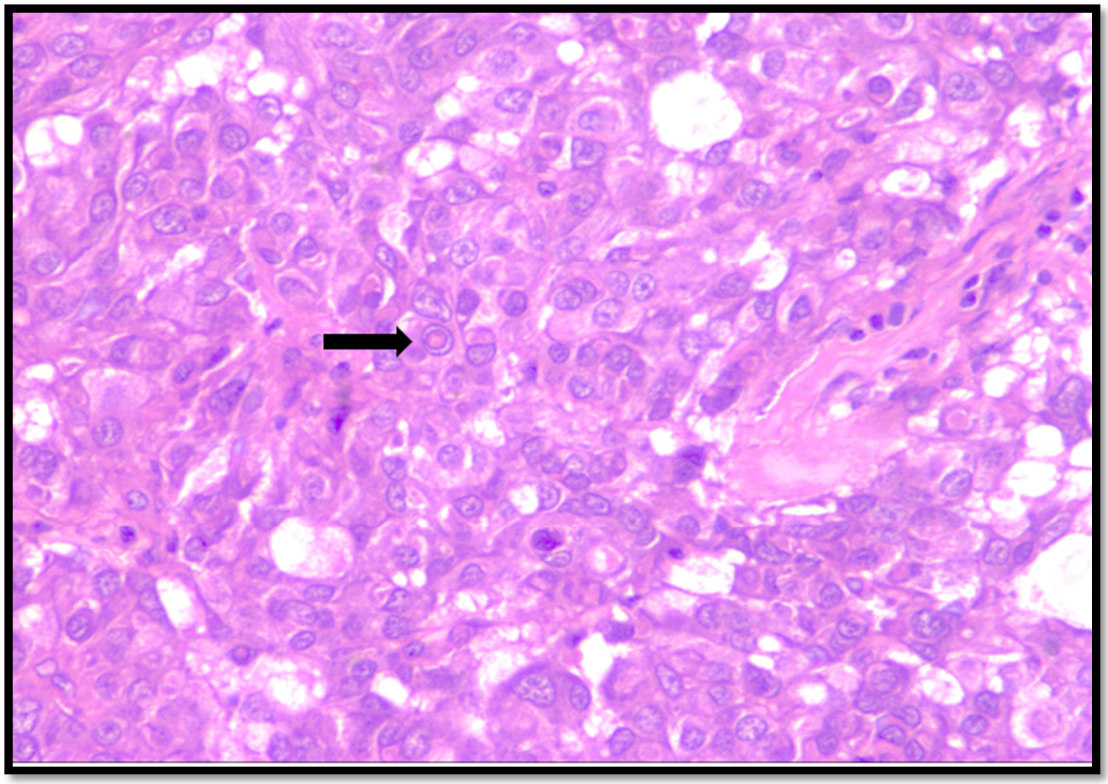
Color plate 7: GH IHC showing cytoplasmic immunoreactivity in Somatotroph adenoma, H & E, 100x



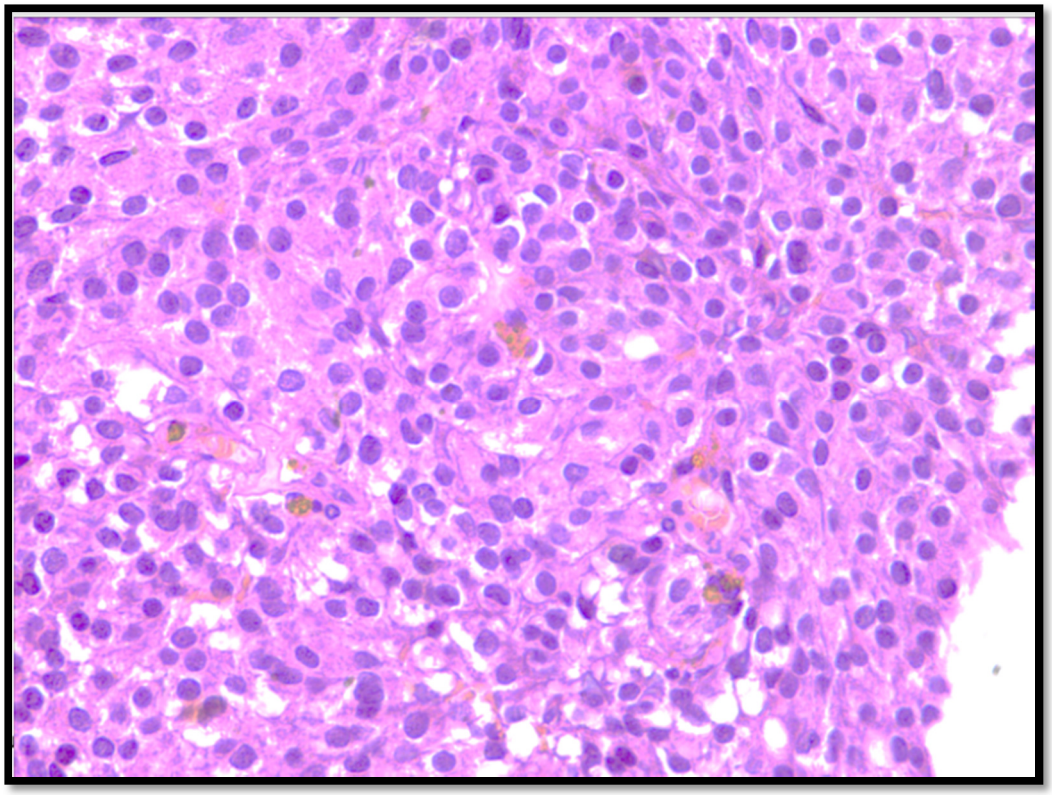
Color plate 8: GH IHC showing cytoplasmic immunoreactivity in Somatotroph adenoma, H & E, 400x



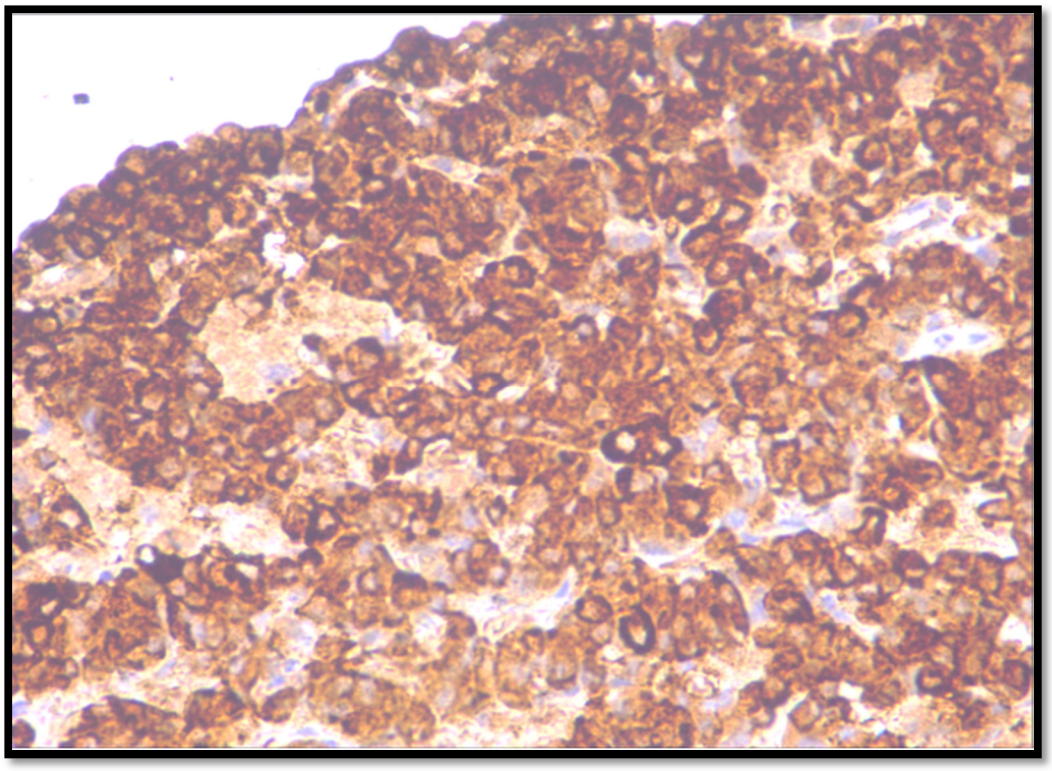
Color plate 9: Pit-1 IHC showing nuclear positivity in Somatotroph adenoma, H & E, 400x



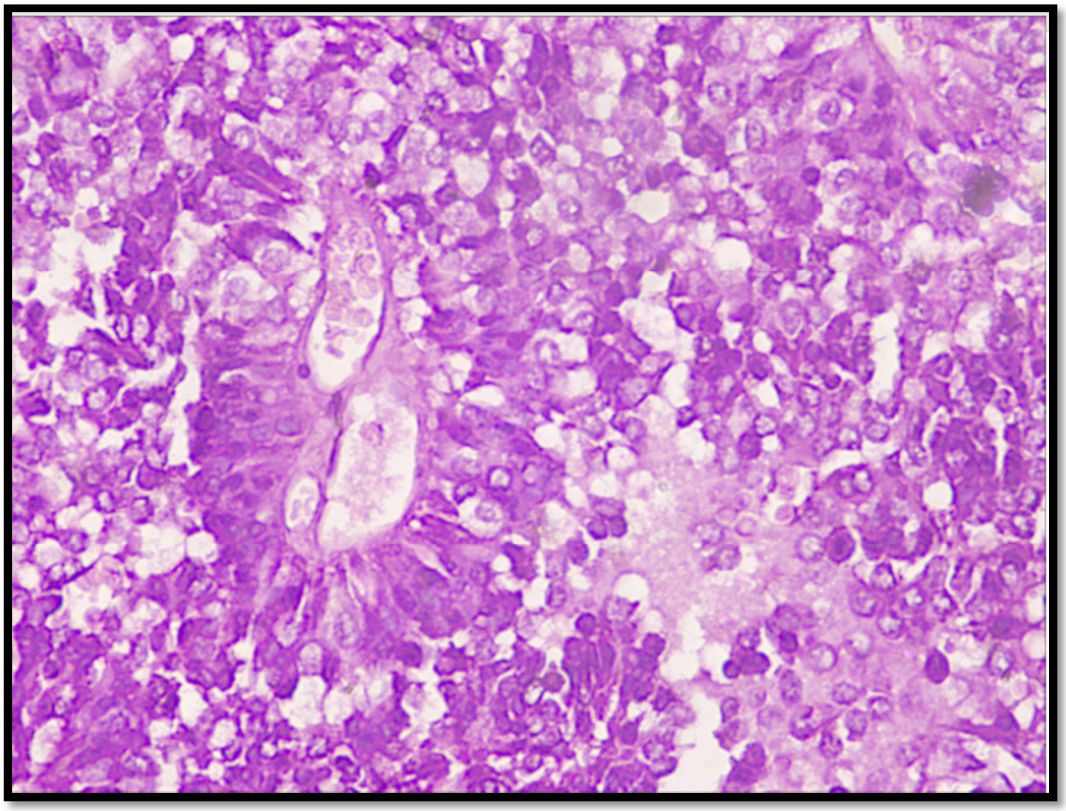
Color plate 10: Prominent intranuclear inclusion in Lactotroph adenoma, H & E, 100x



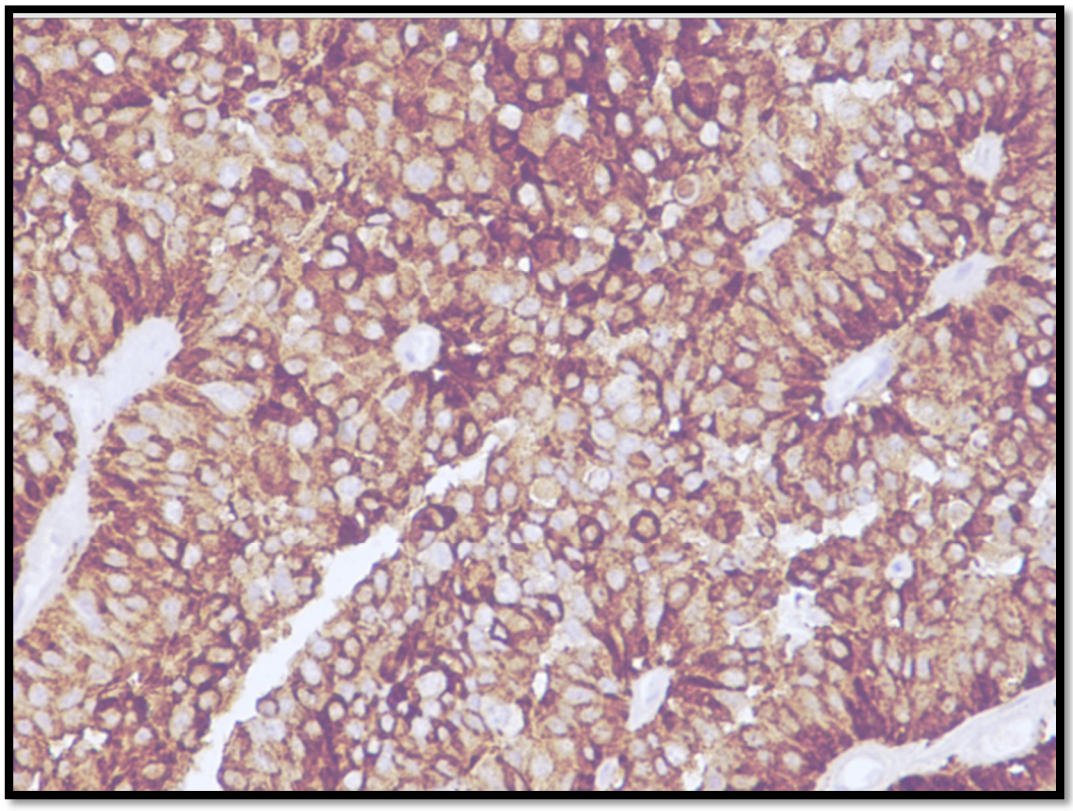
Color plate 11: Lactotroph adenoma, H & E, 400x



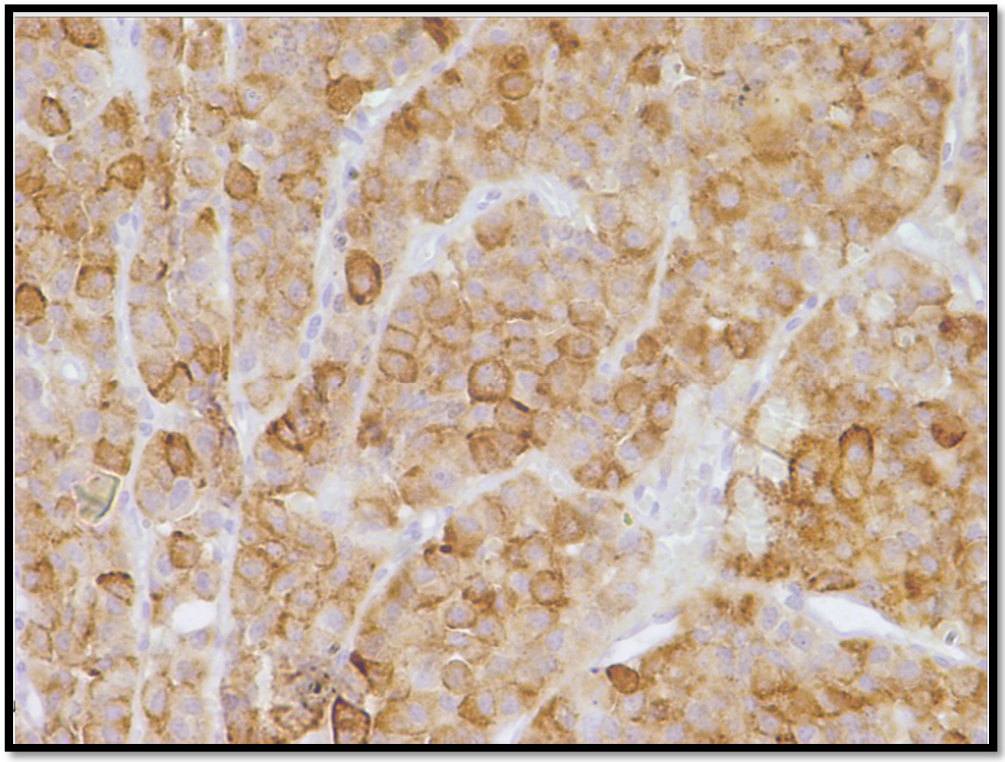
Color plate 12: PRL IHC showing cytoplasmic immunoreactivity in Lactotroph adenoma, 400x



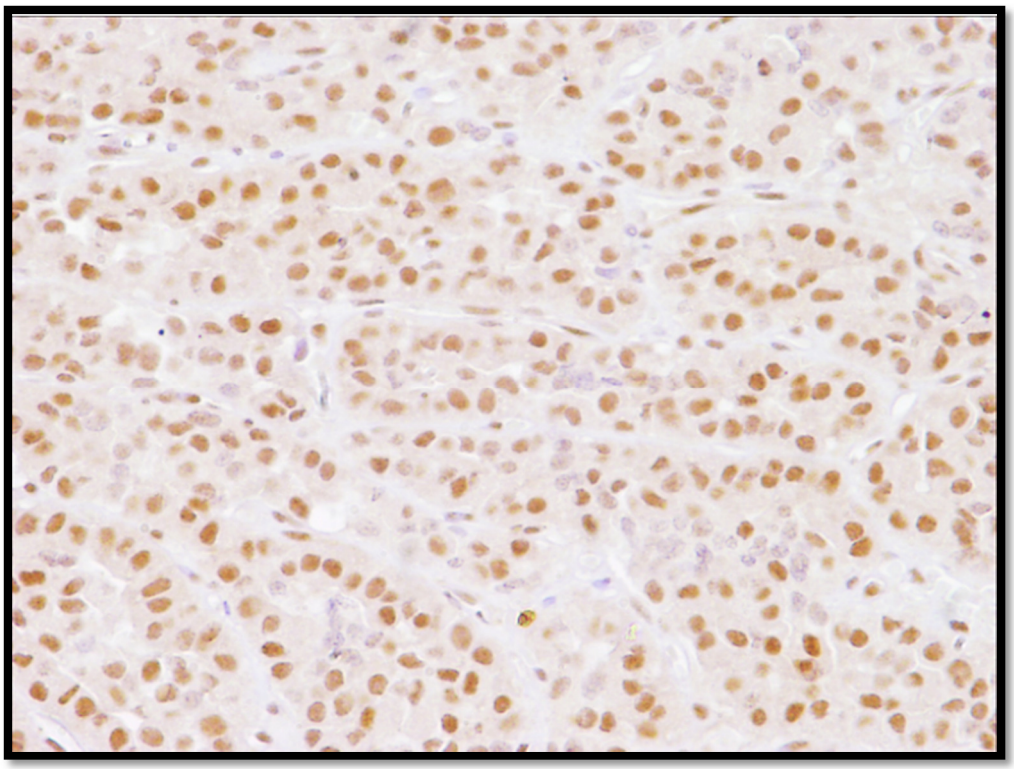
Color plate 13: Perivascular pseudorosettes in Gonadotroph adenoma, H & E, 400x



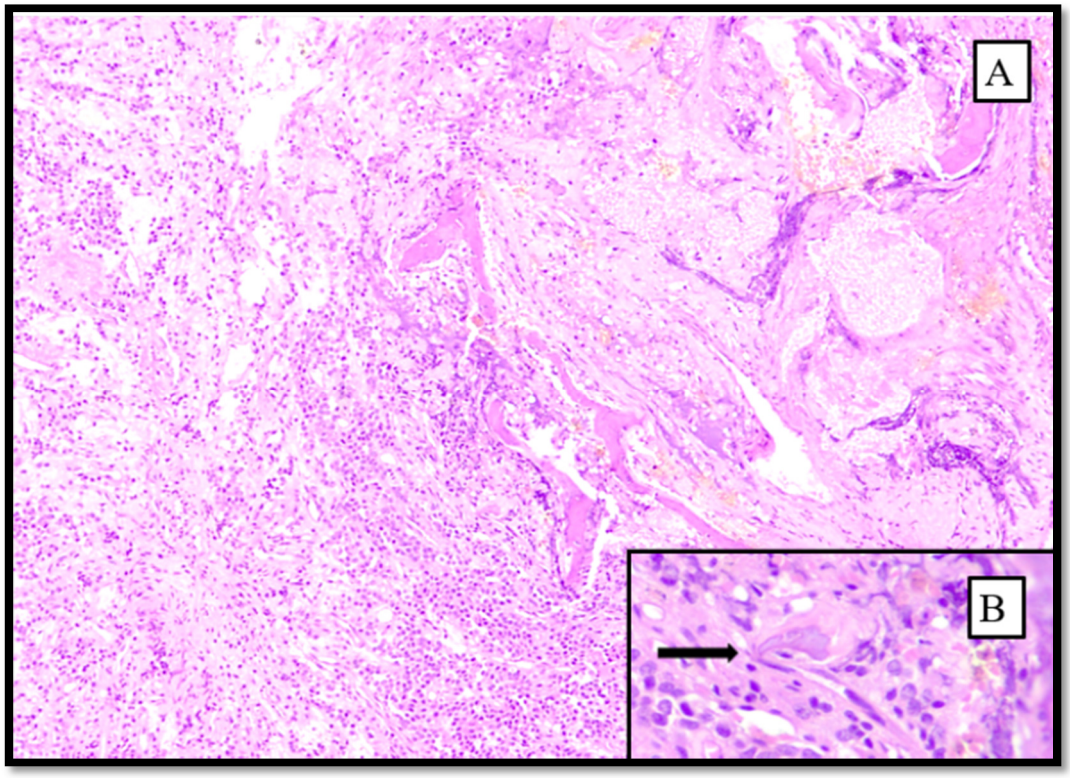
Color plate 14: LH IHC showing cytoplasmic immunoreactivity in Gonadotroph adenoma, 400x



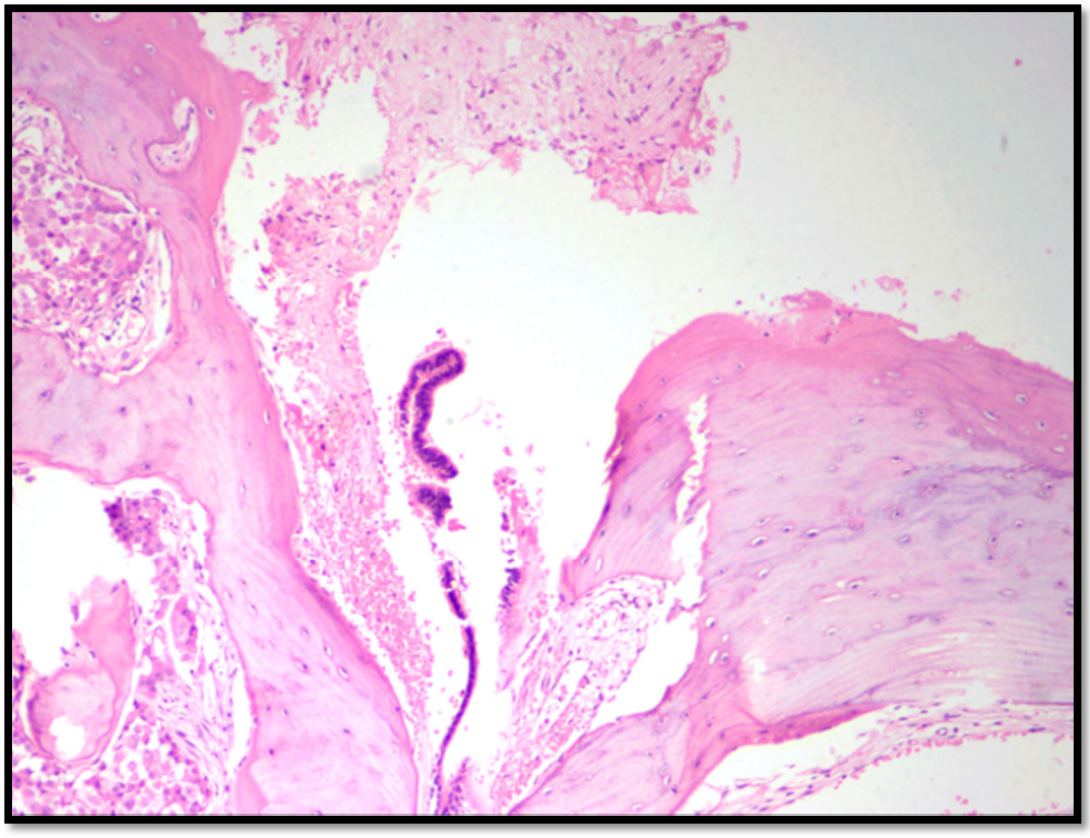
Color plate 15: FSH IHC showing cytoplasmic immunoreactivity in Gonadotroph adenoma, 400x



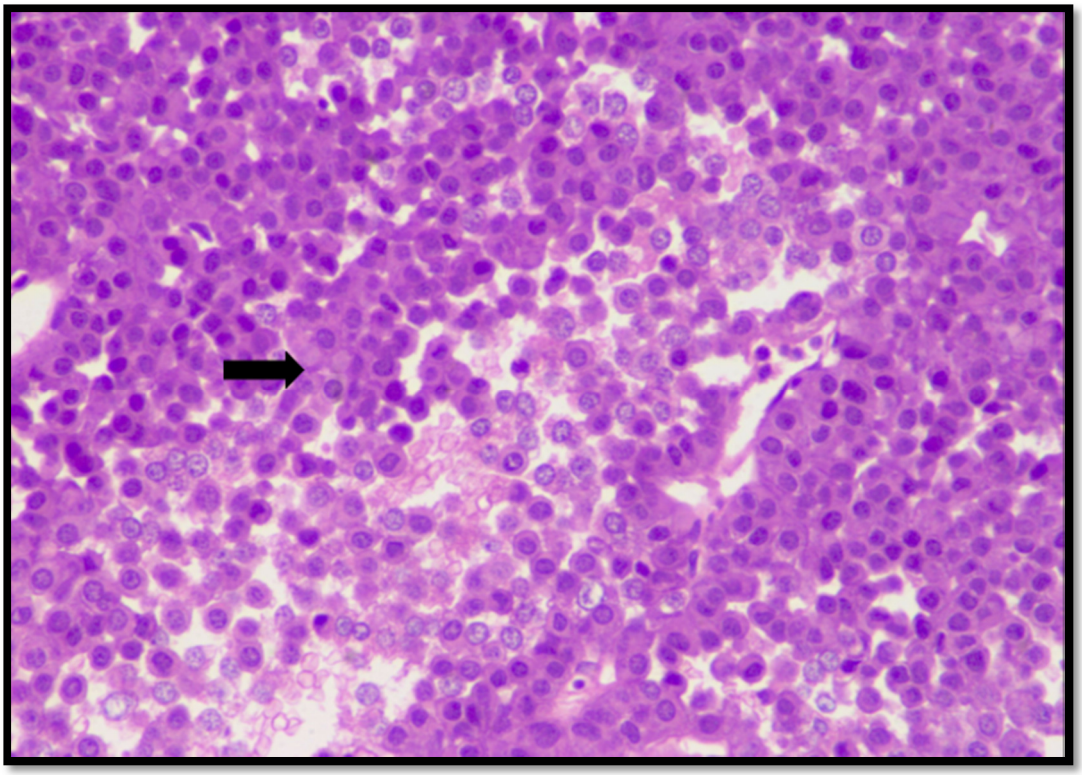
Color plate 16: SF-1 IHC showing nuclear immunoreactivity in Gonadotroph adenoma, 400x



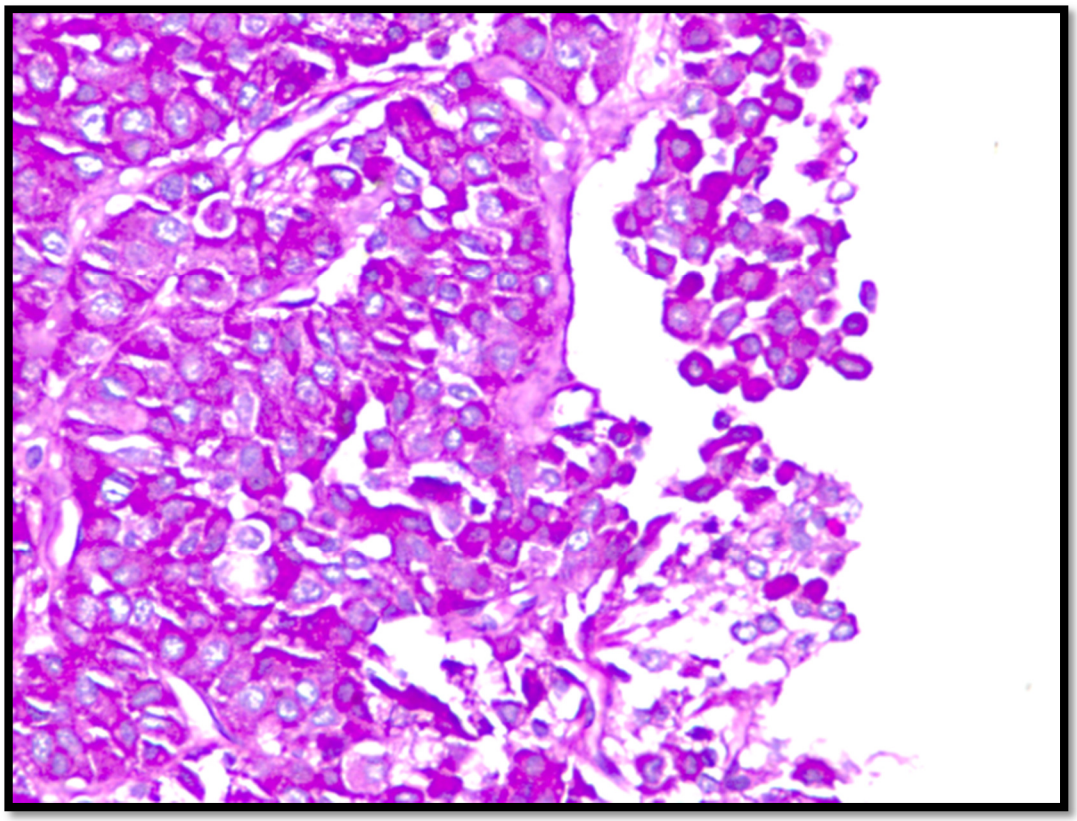
Color plate 17: (A) Pituitary adenoma infiltrating into the surrounding bone, H & E, 100x (B) Inset, 400x



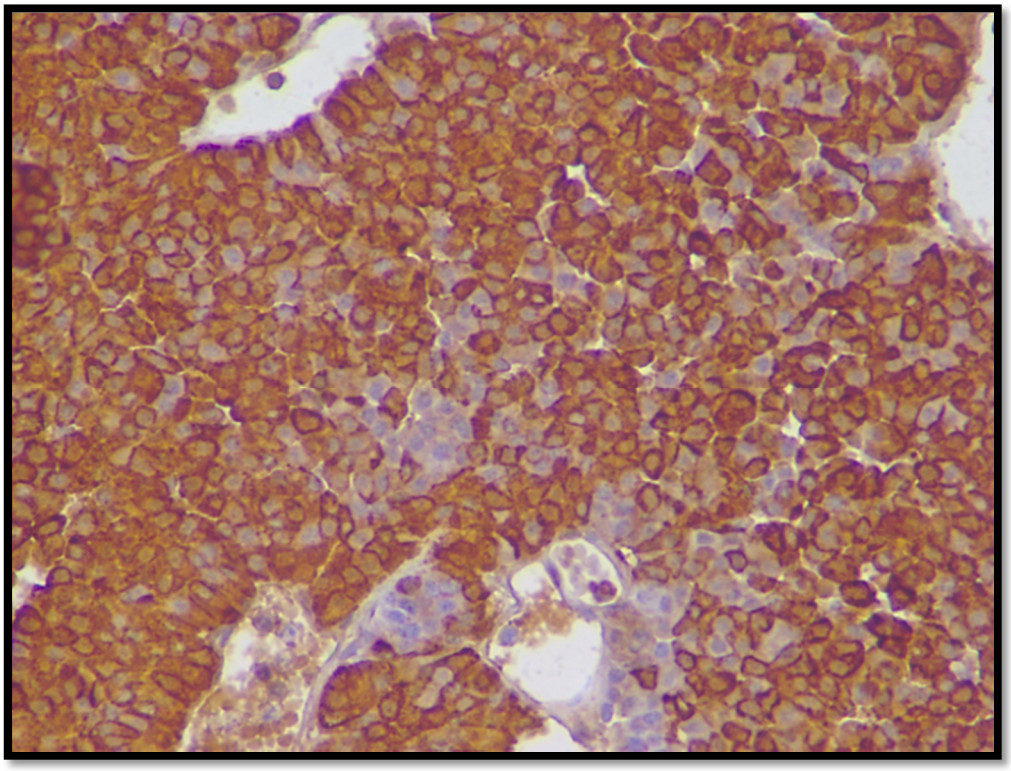
Color plate 18: Hematopoietic marrow and respiratory epithelium, H & E, 100x



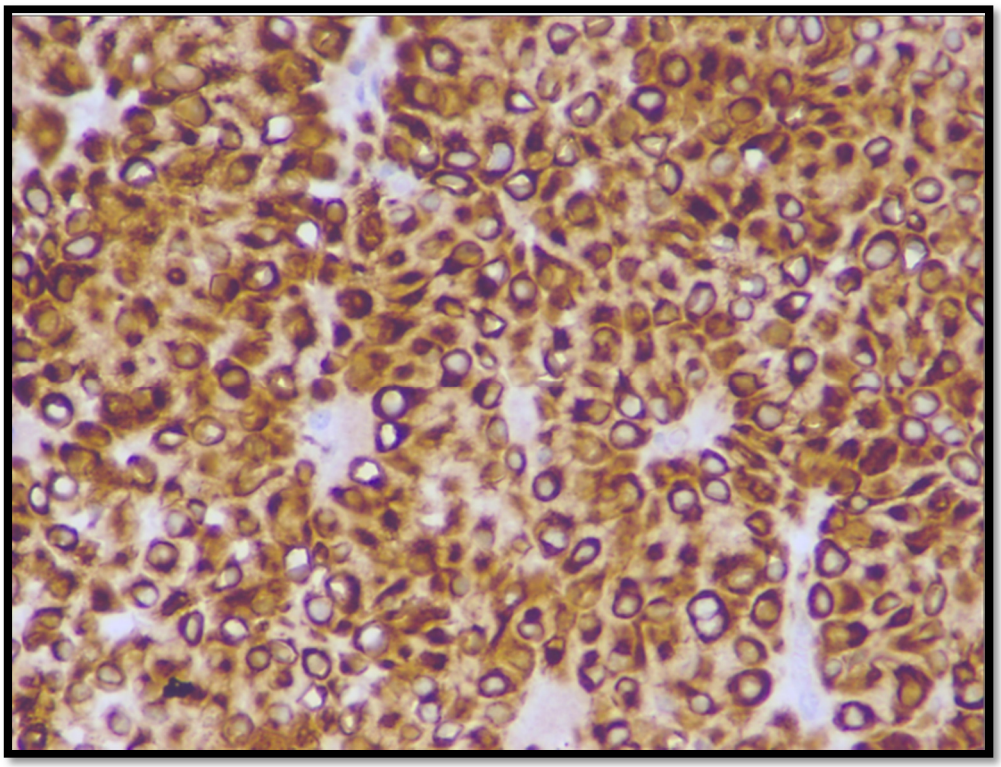
Color plate 19: Hyaline change in Corticotroph adenoma, H & E, 400x



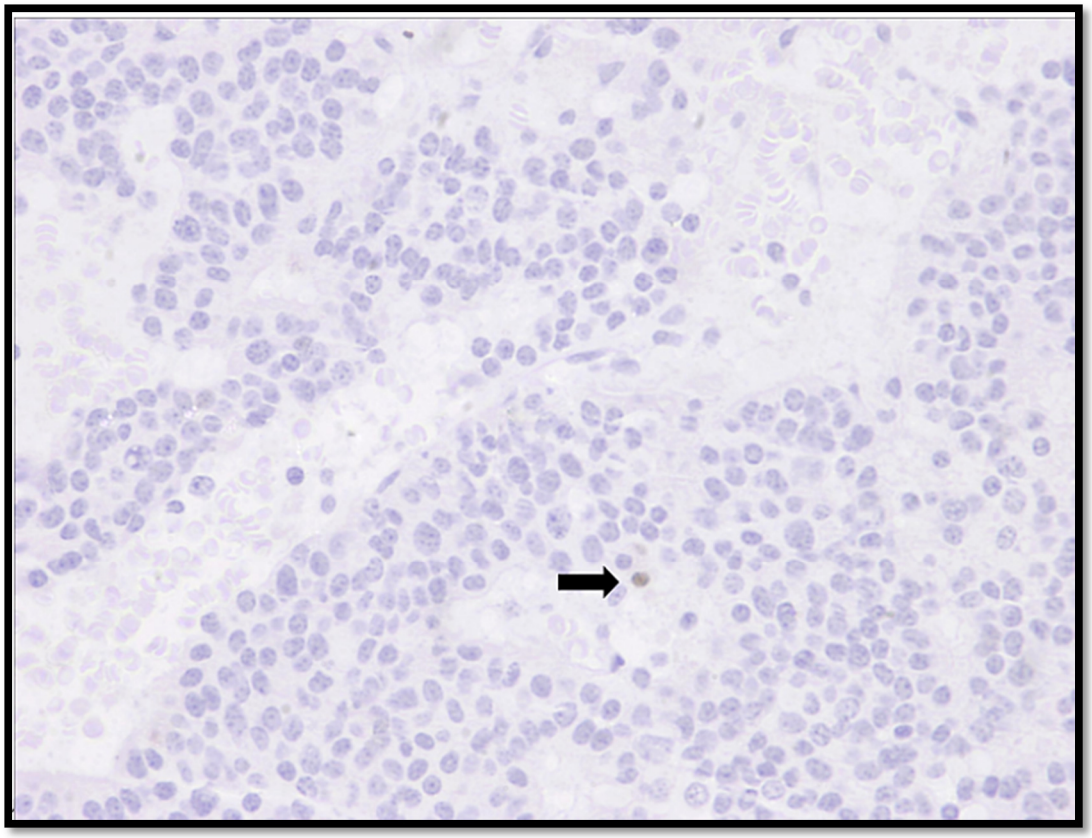
Color plate 20: PAS positivity in Corticotroph adenoma, 400x



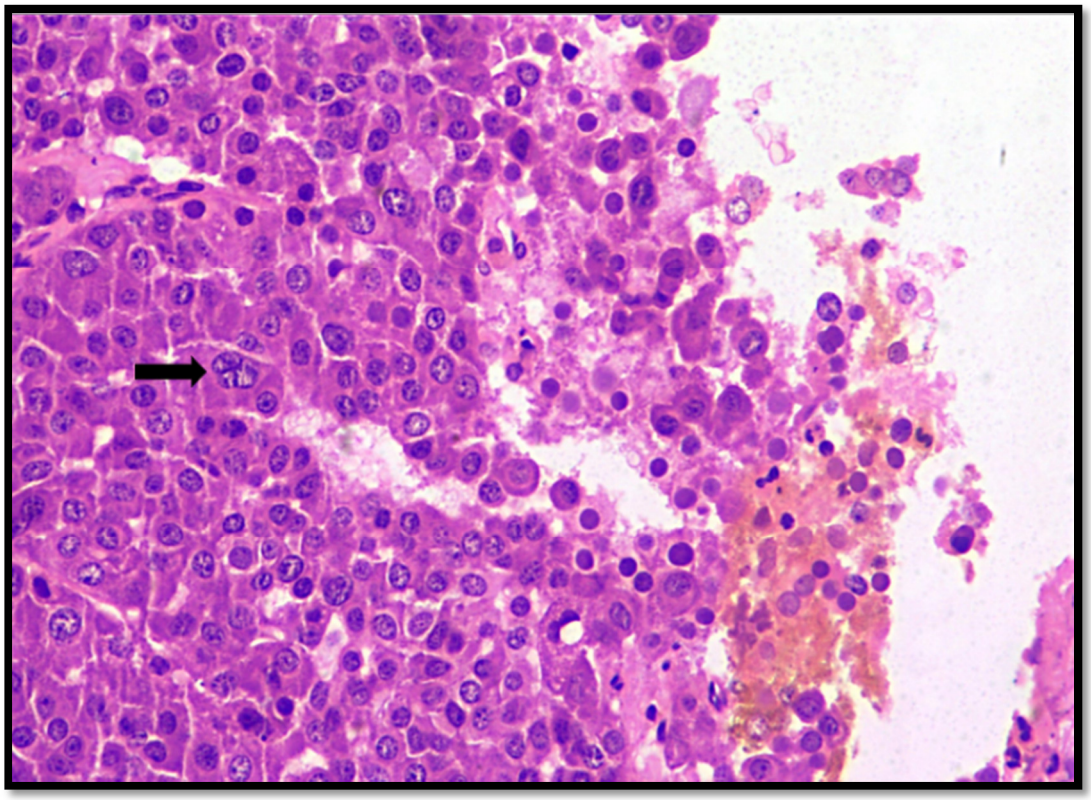
Color plate 21: ACTH IHC showing cytoplasmic immunoreactivity in Corticotroph adenoma, H & E, 400x



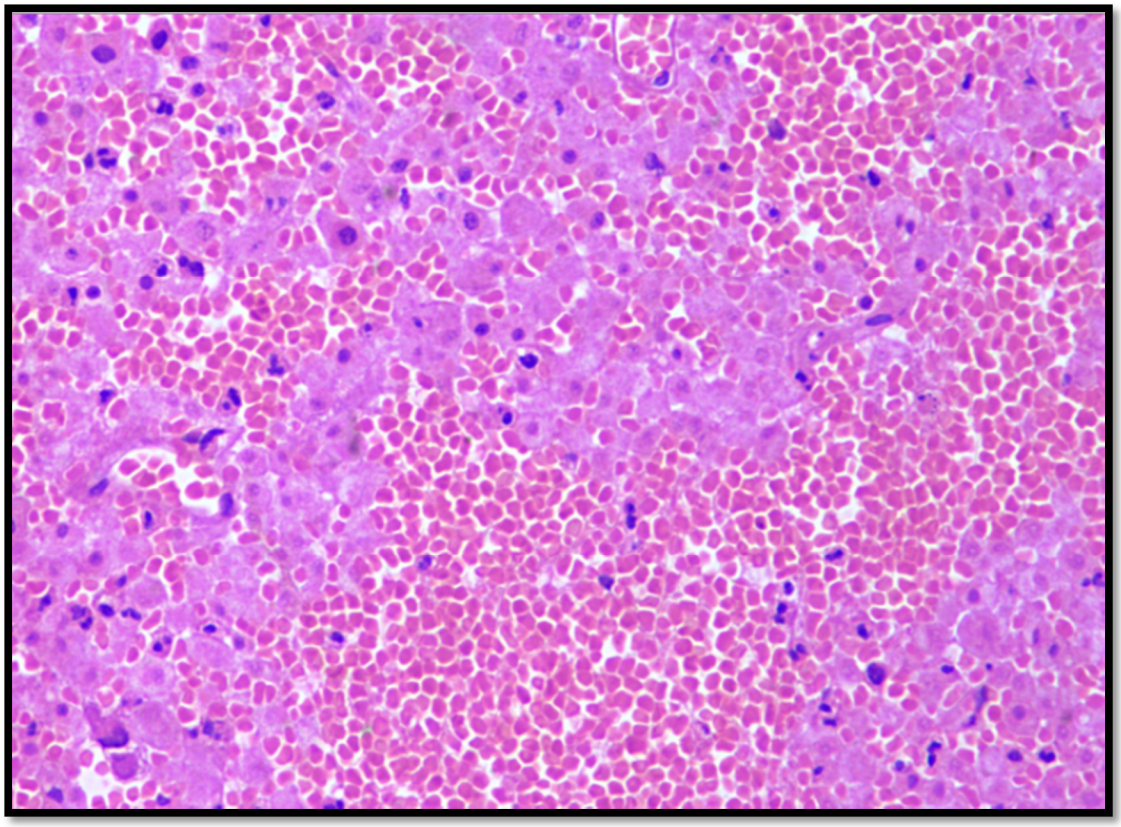
Color plate 22: CAM 5.2 IHC showing ring like positivity in Corticotroph adenoma, 400x



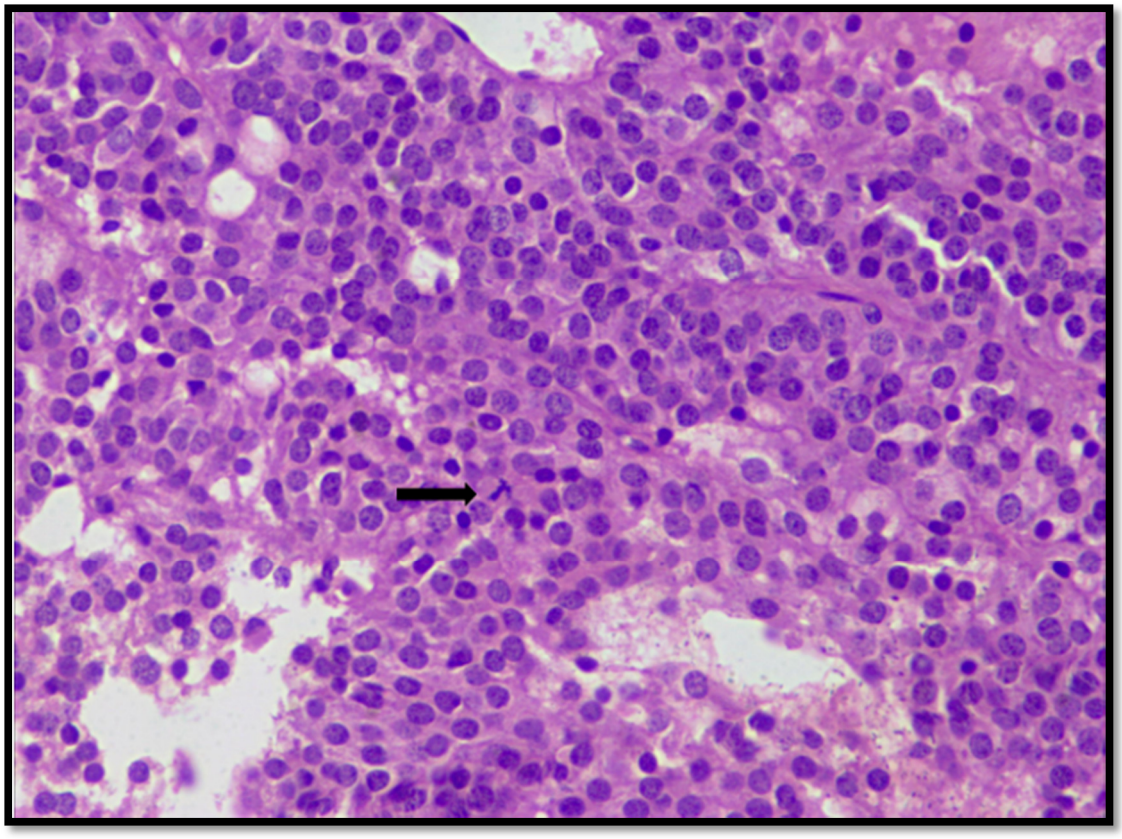
Color plate 23: Ki-67 LI IHC in Pituitary adenoma (<1%) , H & E, 400x



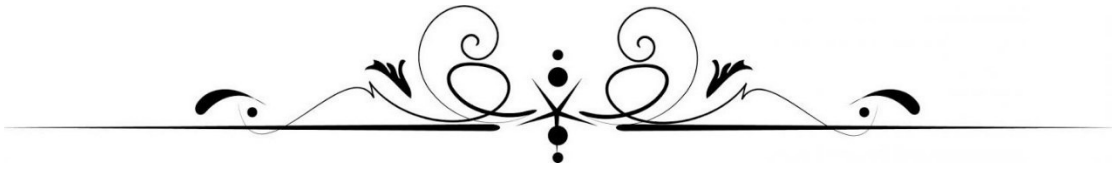
Color plate 24: Focal multinucleation, H & E, 400x



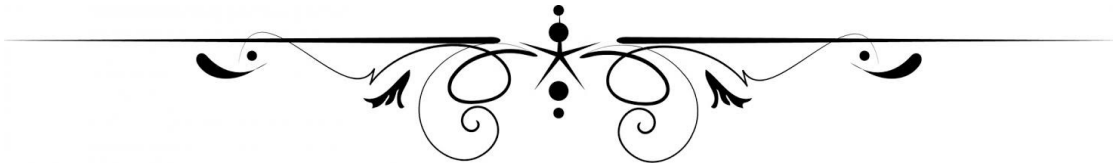
Color plate 25: Pituitary apoplexy, H & E, 400x



Color plate 26: Mitosis, H & E, 400x



OBSERVATIONS & RESULTS



OBSERVATIONS AND RESULTS

The present study is an ambispective (prospective and retrospective) hospital-based observational study conducted at All India Institute of Medical Sciences, Jodhpur from January 2016 to December 2021. A total number of 50 patients with a histopathologically confirmed diagnosis of pituitary adenoma were included in this study.

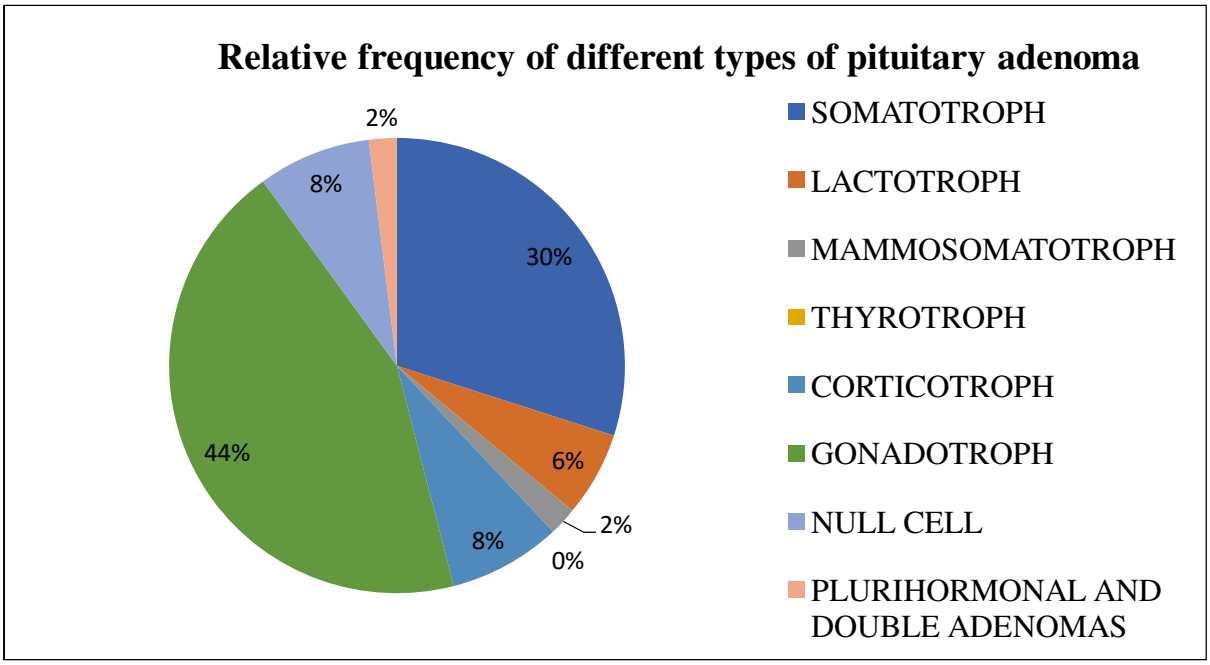


Figure 1: Relative frequency of different types of pituitary adenoma

	Number of cases	Percentage (%)
0-20	2	4
21-30	5	10
31-40	16	32
41-50	16	32
51-60	6	12
61-70	4	8
71-80	1	2
TOTAL	50	100

Table 5: Age distribution of pituitary adenoma

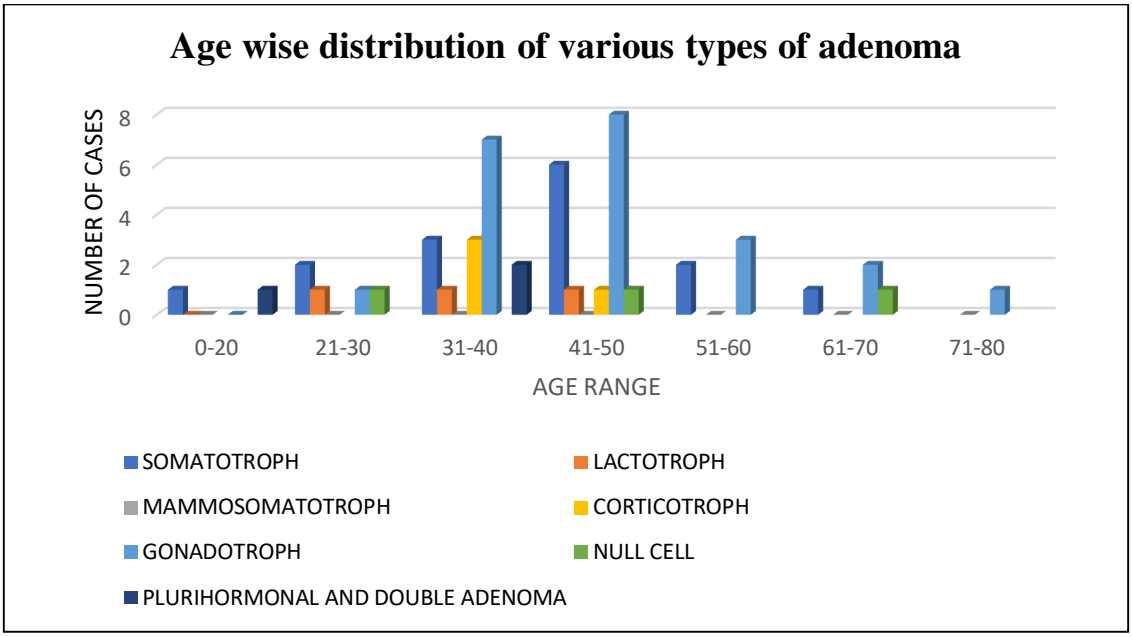


Figure 2: Age wise distribution of different types of pituitary adenoma

The median age in this study was 42 years and the mean age was 42.62 years with a range of 18-77 years. The majority (64%) of patients were in the 3rd and 4th decade with the maximum number of patients (32%) in 31-40 and 41-50 years age group. 2 patients (4%) were below 20 years of age and 1 patient was above 80 years of age (2%).

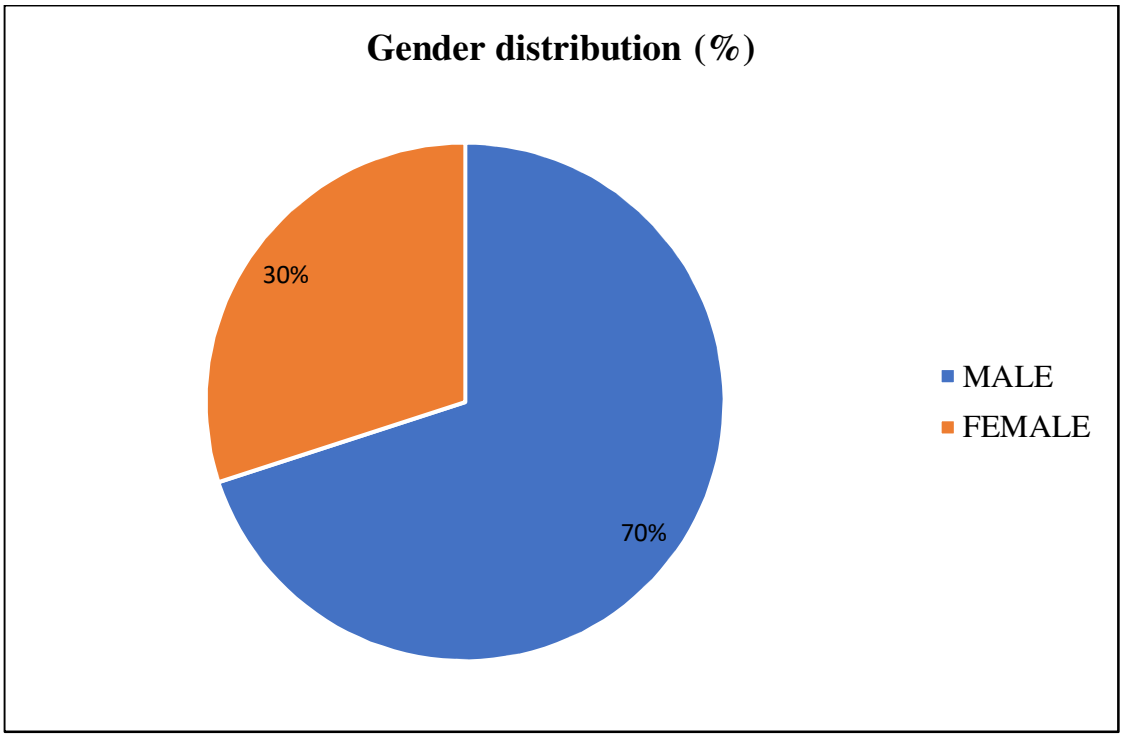


Figure 3: Gender wise distribution

Out of the 50 cases, 35 (70%) patients were male and 15 (30%) patients were female.

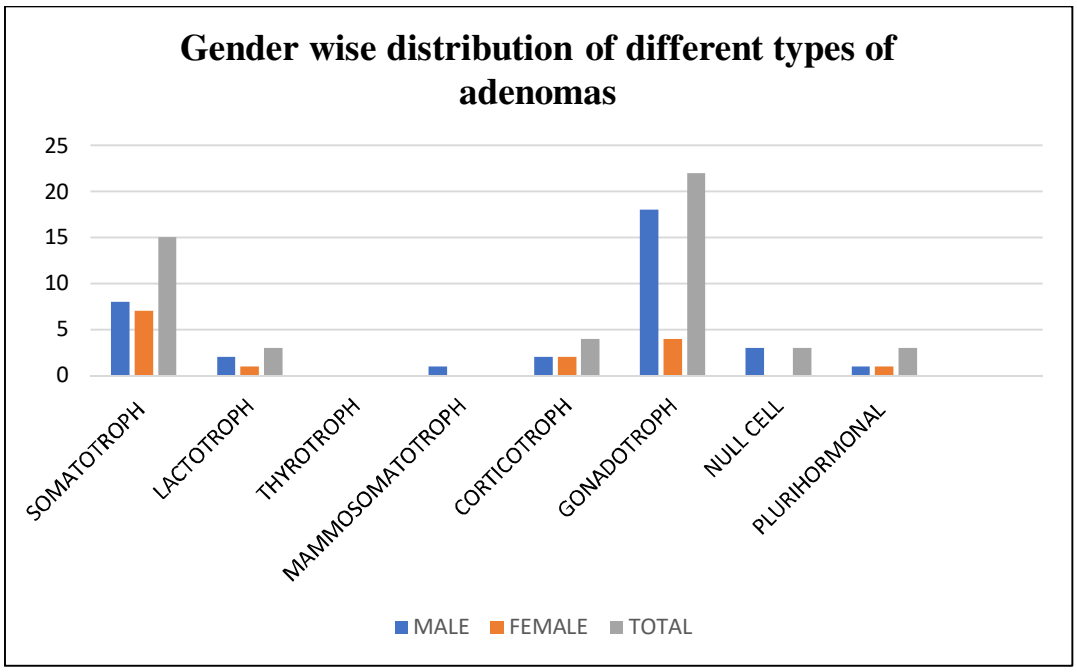


Figure 4: Gender wise distribution of different types of pituitary adenoma

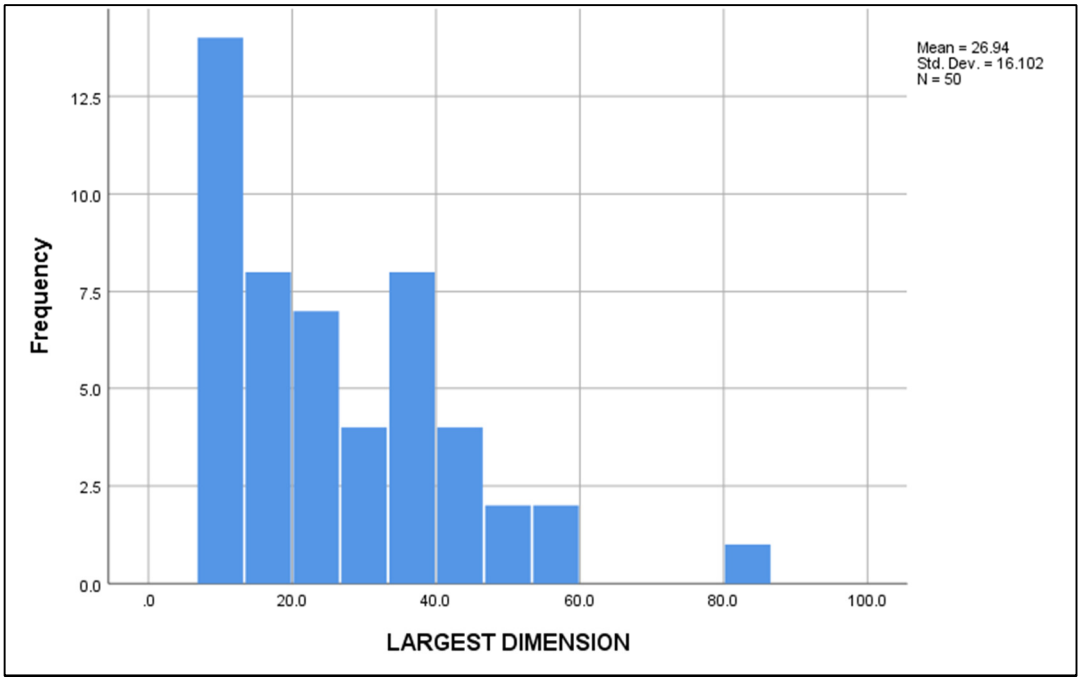


Figure 5: Relative frequency of sizes (mm) of pituitary adenoma

The size of the tumors (in the largest dimension) ranged from 08 mm to the largest 85 mm. The mean size was 26.94mm and the median size was 23.65mm.

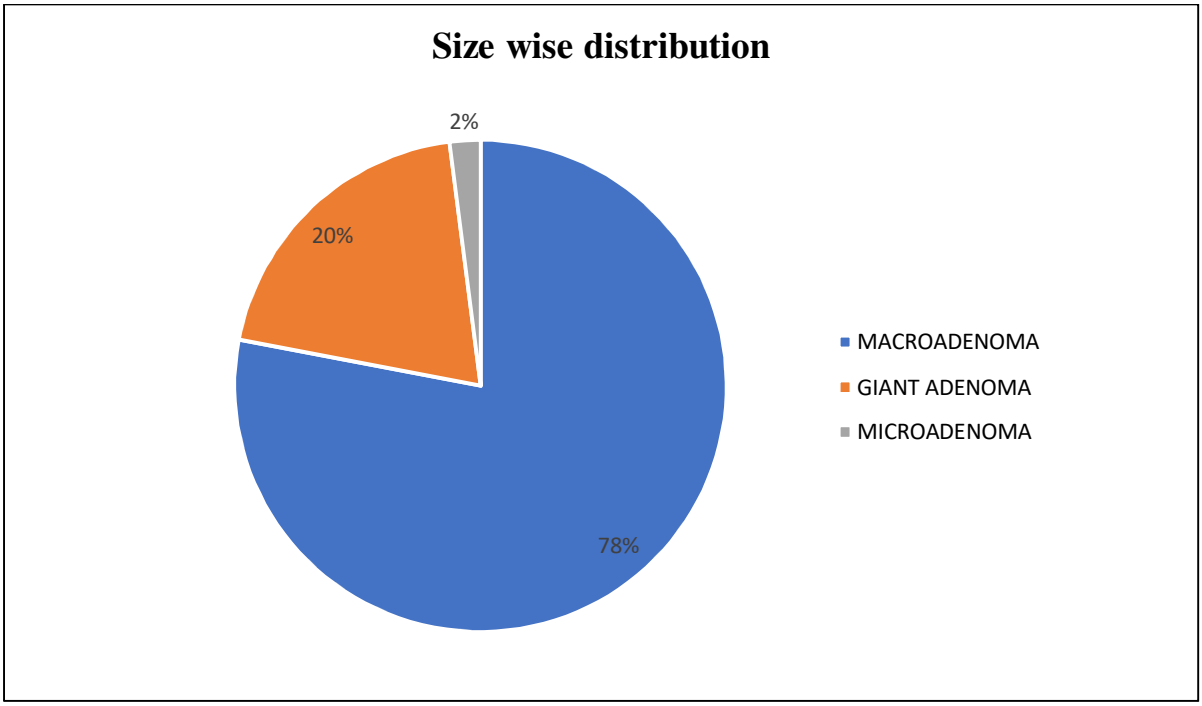


Figure 6: Size wise distribution of pituitary adenoma

Out of the 50 cases, 39 cases (78%) were macroadenoma (>40mm), followed by 10 cases (20%) of giant cell adenoma and 1 case (2%) of microadenoma.

Clinical presentation:

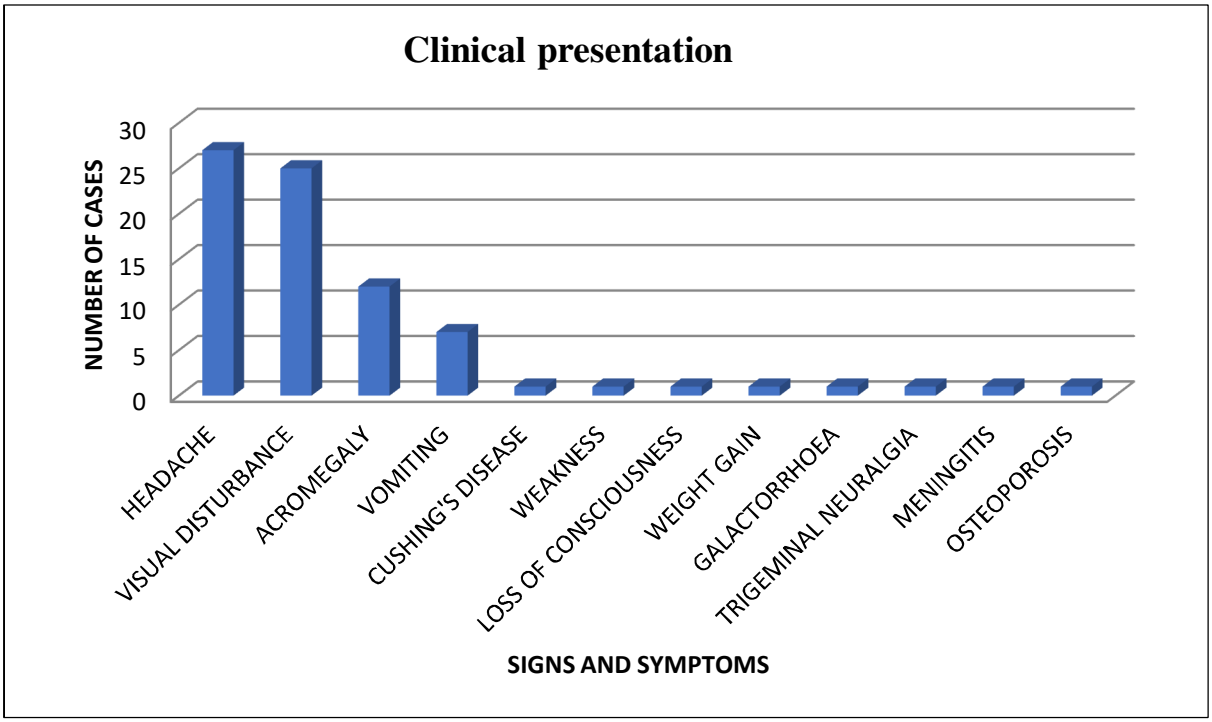


Figure7: Clinical presentation of pituitary adenoma

Clinical details like serum hormonal levels, imaging and presenting complaints were available for 46 patients. 14 cases (28%) were functioning adenomas and 32 cases (64%) were non-functioning adenomas.

Functioning pituitary adenoma (FPA):

Somatotrophic adenoma comprised of the majority of FPA.

Non-functioning pituitary adenoma (NFPA):

Most of the NFPA patients presented with pressure symptoms like headache, followed by visual disturbance, nausea and vomiting. 1 patient presented with trigeminal neuralgia and 1 patient had episodes of seizures.

Apoplexy:

6 of the 50 cases presented with features of pituitary apoplexy.

Features		**Number of cases with apoplexy**
Gender	Male	4
	Female	2
Type of adenoma	Somatotroph adenomas	2
	Gonadotroph adenomas	2
	Corticotroph adenomas	2
Ki-67 Li	<3%	6
Recurrence	Yes	1
Functioning status	Functioning	1
	Non-functioning	5
Size	Macroadenoma	4
	Giant adenoma	2

*Functioning status of 4 adenomas was not available.

Table 6: Cases showing apoplexy

Ki-67 Labeling Index:

The Ki-67 LI ranged from 0% to 4% and the mean was 1.4%.

Statistical analysis found no correlation between size and Ki-67 LI of the adenomas.

Pit-1:

20 out of 50 cases showed nuclear positivity for Pit-1 (Allred score : ≥3). 15 cases were SA, 3 cases were LA, 1 was mammosomatotroph adenoma and 1 was plurihormonal adenoma**.**

	Negative			**Positive**					
Allred score	0	1	2	3	4	5	6	7	8
Number of cases	28	2	0	1	1	1	5	8	4
Total cases	30 (60%)			20 (40%)					

Table 7: Allred scoring in Pit-1 cases

SF-1:

28 out of 50 cases showed nuclear positivity for SF-1 (Allred score : ≥3). 22 cases were GA, 2 cases were LA, 1 was CA, 1 was mammosomatotroph adenoma and 1 was plurihormonal adenoma. 2 cases showed non specific positivity (positive in both nucleus and cytoplasm) and were taken as negative.

	Negative			**Positive**					
Allred score	0	1	2	3	4	5	6	7	8
Number of cases	11	4	7	2	2	0	5	8	11
Total cases	22 (44%)			28 (56%)					

Table 8: Allred scoring in SF-1 cases

Gonadotroph adenoma (GA)

The largest proportion of our cases comprised of gonadotrophic adenomas. A total number of 14 cases (28%) were either immunopositive for β-LH or β-FSH and β-LH both. All of these cases were positive for SF-1.

	SF-1 Positive	SF-1 Negative	Total
β-LH Positive	14	0	14
β-FSH+ β-LH Positive	10	0	10
Hormonal Markers Negative	8	0	8

Table 9: SF-1 positivity in gonadotroph adenoma

Additionally, 8 cases (16%) of GA were immunonegative for all the hormonal markers but showed strong (Allred score ≥3) positive nuclear expression of SF-1. These cases were considered to be silent gonadotroph adenoma (SGA).

So, a total number of 22 (44%) cases were considered as gonadotrophic adenoma.

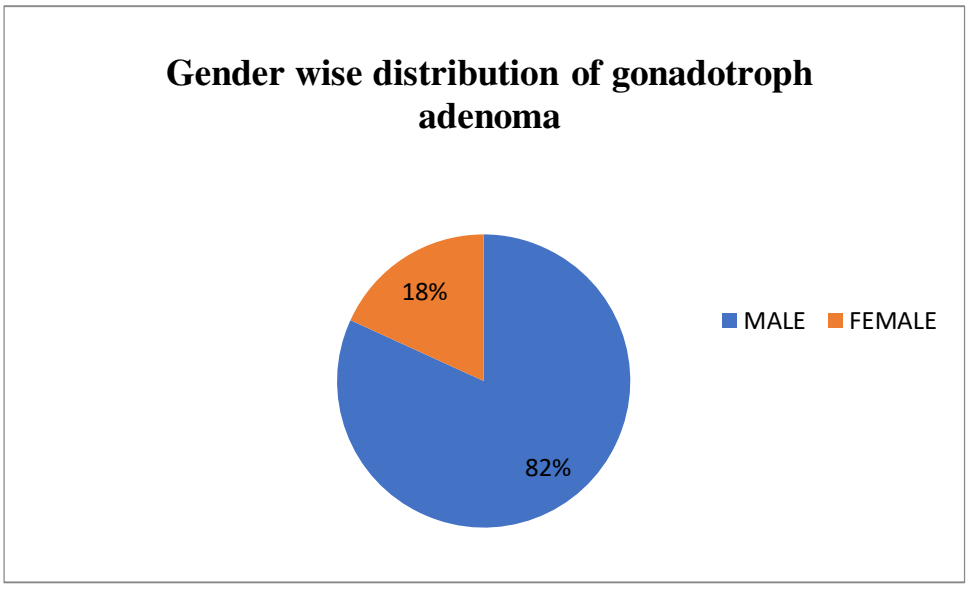


Figure 8: Gender wise distribution of gonadotroph adenoma

Of the 22 GA cases, 18 were males and 4 were females.

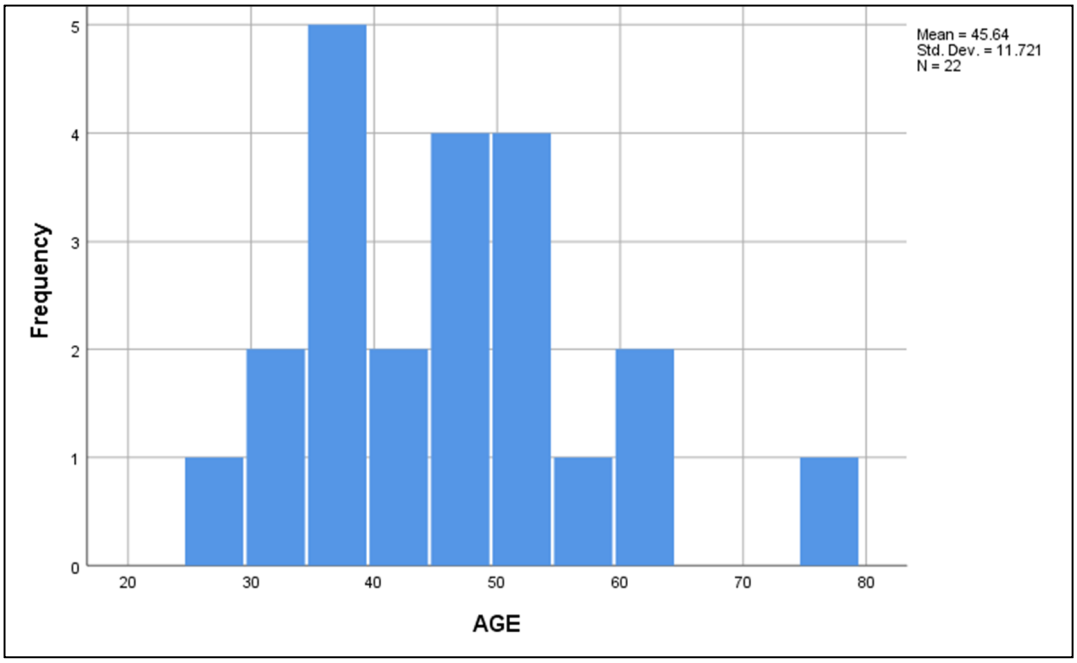


Figure 9: Age wise distribution of gonadotroph adenoma

The mean age of gonadotroph adenoma was 45.64 years and the age range was from 27 years to 77 years.

Size :

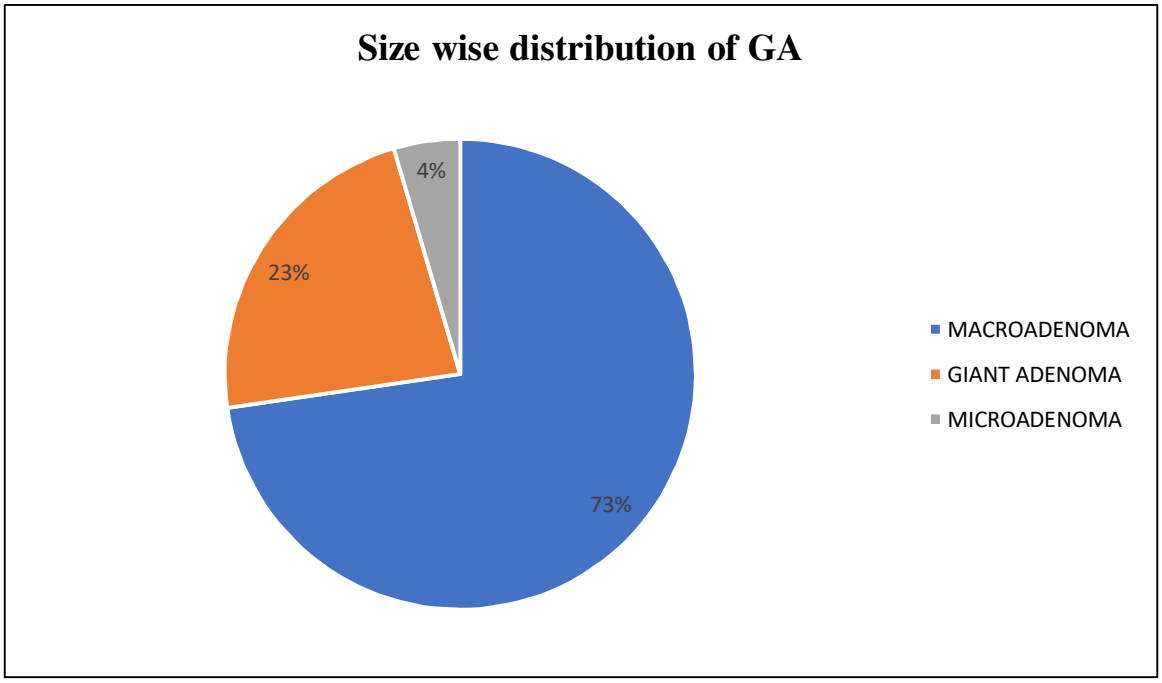


Figure 10: Size wise distribution of gonadotroph adenoma

The size (in largest dimension) ranged from 8mm to the largest 85mm, the mean size being 32.45mm. There were 16 macroadenomas, 5 giant adenomas and 1 microadenomas.

Recurrence :

In GA, 3 were recurrent adenomas and 11 were non-adenomas.

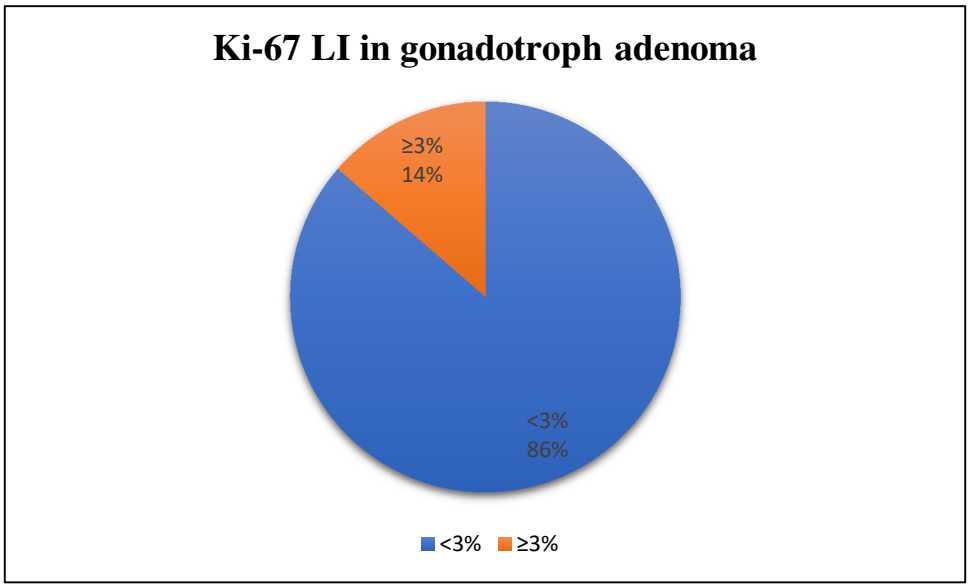


Figure 11 : Ki-67 LI in gonadotroph adenoma

The Ki-67 LI ranged from <1% to 3%, the mean Ki-67 LI being 1.53%.

Microscopy:

Specimens were stained with reticulin for assessing the adenomatous area/ foci, which predominantly showed disruption of reticulin meshwork. The tumor was arranged in diffuse sinusoidal arrangement to perivascular pseudorosetting arrangement. The individual cells were small to medium sized and polygonal in shape. The cytoplasm was chromophobic and showed minimal nuclear atypia. All of the cases showed strong nuclear positivity for SF-1 IHC, while none was positive to Pit-1 IHC.

Statistical analysis revealed that β-FSH has positive correlation with SF-1 (Spearman's ρ= .443; p<0.001), whereas, it has significant negative correlation with Pit-1 (Spearman's ρ= -.391; p<0.001). β-LH has positive correlation with SF-1 (Spearman's ρ= .553; p<0.001), whereas, it has significant negative correlation with Pit-1 (Spearman's ρ= -.488; p<0.001).

Somatotroph adenoma (SA)

Of all the PA examined, 17 cases (34%) showed diffuse cytoplasmic positivity for Growth hormone (GH) IHC.

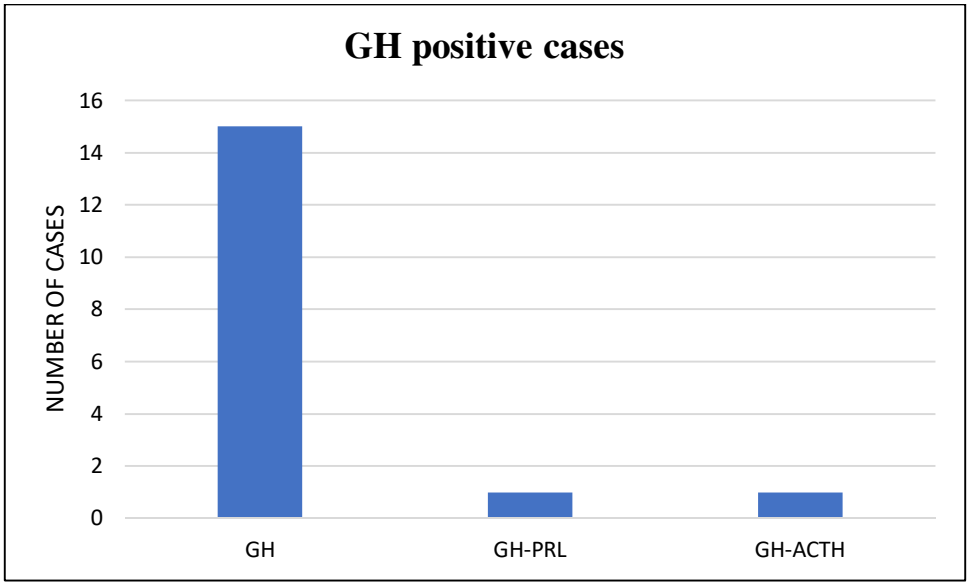


Figure 12: GH positive cases

Out of these 17 cases, one case was positive for PRL and one for ACTH.

A total number of 15 (30%) adenoma did not show any additional positivity for other hormonal IHCs. 15 cases (30%) were categorized as pure somatotroph adenomas.

Gender:

Of 15 cases, 8 were males and 7 were females.

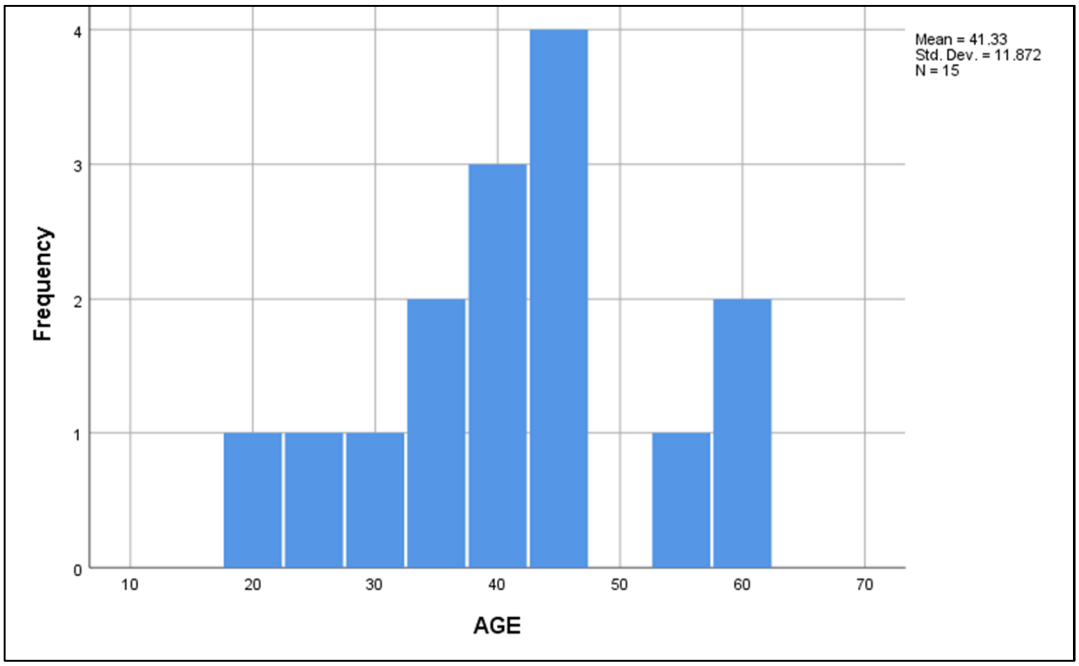


Figure 13: Age wise distribution of somatotroph adenoma

The mean age for somatotroph adenoma was 41.33 years and the age range was from 20 years to 62 years.

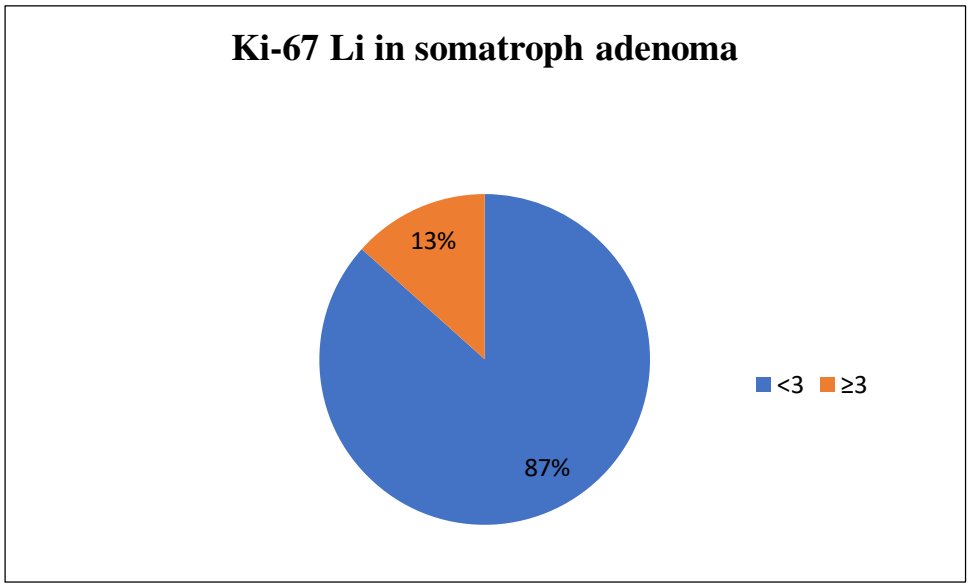


Figure 14: Ki-67 Li in somatotroph adenoma

Ki-67 Li in the somatotroph adenoma ranged from 1% to 4%, the mean Ki-67 Li being 1.4%.

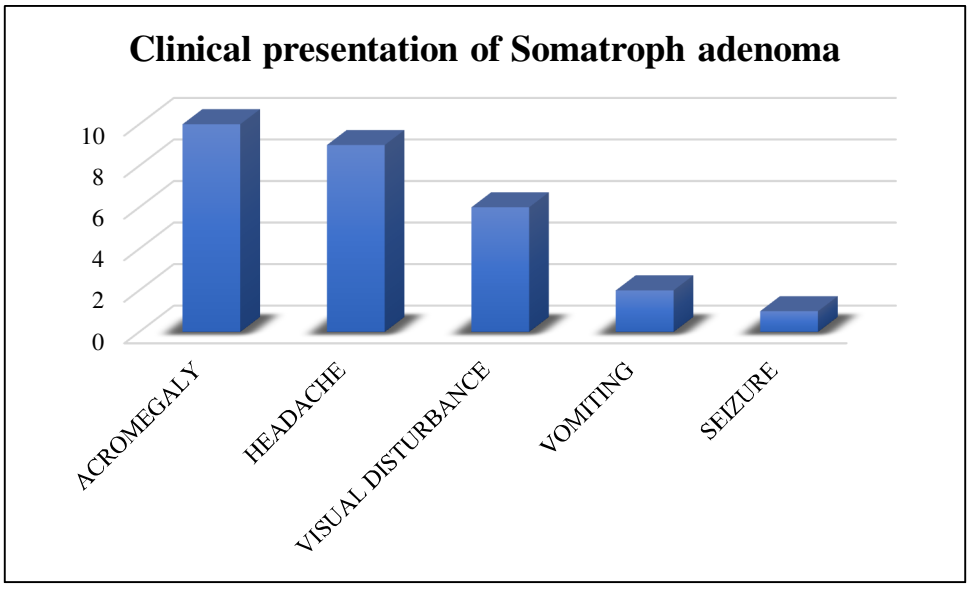


Figure 15: Clinical presentation of somatotroph adenoma

Details of the clinical presentation were present in all of the cases. Most of the patients presented with features of acromegaly (10 cases), followed by pressures symptoms like

headache (9 cases), visual disturbance (6 cases) and vomiting (2 cases). One patient also presented with seizures.

Biochemically, 13 of the 15 cases presented with an increased serum concentration of IGF-1 or serum GH levels and 2 cases presented with normal serum concentrations of IGF-1 and GH.

Size wise distribution of SA:

The size (in the largest dimension) ranged from 10mm to the largest 56mm, the mean size being 25.93mm. Eleven cases were macroadenomas and 4 cases were giant adenomas.

Recurrence of SA:

Only one of the cases was a recurrent adenoma.

Microscopy:

Specimens examined were stained with reticulin for assessing the adenomatous area/ foci, which predominantly showed disruption of reticulin meshwork. The cells were predominantly monomorphic and round to oval with eosinophilic cytoplasm. An important characteristic feature was the presence of round to spherical fibrous bodies and showed pale staining with H & E. Pan-CK or CAM 5.2 IHC revealed perinuclear staining of these fibrous bodies.

These tumors showed diffuse cytoplasmic staining for GH IHC and all of our cases were nuclear positive for Pit-1 IHC. Two cases also showed nuclear positivity for SF-1.

	Total number of cases	**Percentage (%)**
Pit-1 Positive	15	100
Pit-1 Negative	0	0
SF-1 Positive	2	13.3
SF-1 Negative	13	86.6

Table 10: Proportion of Pit-1 and SF-1 positivity in somatotroph adenoma

Statistical analysis revealed that GH has positive correlation with Pit-1 (Spearman's ρ= .830; p<0.001), whereas, it has significant negative correlation with SF-1 (Spearman's ρ= -.555 ;p<0.001).

Corticotroph adenoma (CA)

Of all the pituitary adenomas examined, 5 (10%) showed diffuse cytoplasmic positivity for ACTH.

	ACTH	**GH-ACTH**
Positive Cases	4/50 (8%)	1/50 (2%)

Table 11: ACTH positive cases

Out of the five ACTH positive cases, one case was also positive for GH.
4 of the ACTH positive adenomas did not show additional positivity for any of the other hormonal IHC. So, the total number of pure corticotroph adenoma were 4 (8%) of 50 cases.

Gender:
In Corticotroph adenomas, there was 1 male patient and 3 were females.

Age:
The mean age for corticotroph adenoma was 41.25 years and the age range was from 35 years to 53 years.

Ki-67 Li:
Ki-67 Li in the corticotroph adenoma ranged from 0% to 2%, mean Ki-67 Li being 1.5%.

Clinical features :

1 of the 4 patients had Cushing's disease with had elevated cortisol levels. Three patients presented with pressure symptoms, one of which had galactorrhoea and elevated prolactin levels. One patient also had hypothyroidism.

Size :
The size (in the largest dimension) ranged from 10mm to the largest 17mm, the mean size being 14mm and all corticotroph adenoma were macroadenoma.

Recurrence :

None of the cases recurred.

Microscopy:

Specimens examined were stained with reticulin for assessing the adenomatous area/ foci, which predominantly showed disruption of reticulin meshwork. The tumor cells were arranged in diffuse sheets to a few showing perivascular pseudorosettes. The individual cells were basophilic and PAS-positive and had coarse chromatin and conspicuous nucleoli. Few multinucleated cells were noted. CAM 5.2 IHC showed ring-like cytoplasmic expression.

None of the four cases were positive for Pit-1 IHC.

Of all the corticotroph adenoma cases, 1 case was positive for SF-1 and 3 cases were negative for SF-1.

Statistical analysis showed no significant correlation between ACTH with Pit-1 and SF-1.

Lactotroph adenoma (LA)

Of all the PAs examined, 4 cases (8%) showed diffuse cytoplasmic positivity for Prolactin (PRL) IHC.

	PRL	**GH-PRL**
Positive cases	3/50 (6%)	1/50 (2%)

Table 12: PRL positive cases

Out of the 4 cases, one case was positive for GH as well (GH-PRL= 2%). A total number of 3 (6%) adenoma did not show additional positivity for any of the other hormonal IHC. So, the total number of pure lactotroph adenoma was 3 (6%).

Gender:

Of 3 cases, 2 were males and 1 was female.

Age:

The mean age for lactotroph adenoma was 42.67 years and the age range was from 21years to 50 years.

Ki-67 Li:

Ki-67 Li in the lactotroph adenoma ranged from <1% to 3%, mean Ki-67 Li being 1.66%.

Clinical presentation:

Clinical presentation details were available in 2 of the 3 cases. One patient presented with visual disturbance and the other with osteoporosis and meningitis.

Size:

The size (in the largest dimension) ranged from 10mm to the largest 40mm, the mean size being 20mm. Two of the cases were macroadenoma and one case was a giant adenoma.

Microscopy:

Specimens examined were stained with reticulin for assessing the adenomatous area/ foci, which predominantly showed disruption of reticulin meshwork. The tumors were arranged in diffuse sheets and trabecular patterns. The cells were predominantly monomorphic, were round to oval with eosinophilic to chromophobic cytoplasm. One case had co-existent meningioma showed prominent intranuclear inclusions.

Three cases were positive for Pit-1 and 2 cases were positive for SF-1.

Statistical analysis revealed that PRL has positive correlation with Pit-1 (Spearman's $\rho = .288$; $p = 0.042$), whereas, it has significant negative correlation with SF-1 (Spearman's $\rho = -.107$; $p = 0.458$).

Mammosomatotroph adenoma

One case (2%) showed diffuse cytoplasmic positivity in both GH and PRL hormones.

This 18 years old patient presented with acromegaly and headache and serum hormonal levels for both Growth hormone and Prolactin were raised. This macroadenoma measured 25mm in the largest dimension. The Ki-67 LI was 3%.

Microscopy:

The specimen examined was stained with reticulin for assessing the adenomatous area/ foci, which predominantly showed disruption of reticulin meshwork. The cells were monomorphic, arranged in diffuse sheets, predominantly eosinophilic and were positive for both GH and PRL IHC.

The tumor cells showed nuclear positivity for Pit-1 and negative for SF-1.

Plurihormonal and double adenomas

One case (2%) showed cytoplasmic positivity for both GH-ACTH and was taken as plurihormonal adenoma. This 39 year old male patient presented with acromegaly, vomiting and headache and had increased serum IGF-1 levels.

Ki-67 LI for this case was 2% and the tumor was a macroadenoma (measured 12mm in largest dimension).

There was no history of recurrence.

Microscopy:

Specimen examined was stained with reticulin for assessing the adenomatous area/ foci, which predominantly showed disruption of reticulin meshwork. The tumor cells were arranged in sheets and had chromophobic cytoplasm. They showed diffuse immunopositivity for both GH and ACTH IHCs.

This case showed nuclear positivity for both Pit-1 and SF-1.

Null Cell Adenoma

Four of the cases (8%) were negative for all the hormonal and transcription factor IHC markers. The biochemical hormonal assay was also negative for all four cases. These cases were considered as Null cell adenoma. All the cases were male and the mean age was 43.75 years and the age range was from 27 years to 68 years.

The Ki-67 LI ranged from 0% to 2% and the mean Ki-67 LI was 1%.

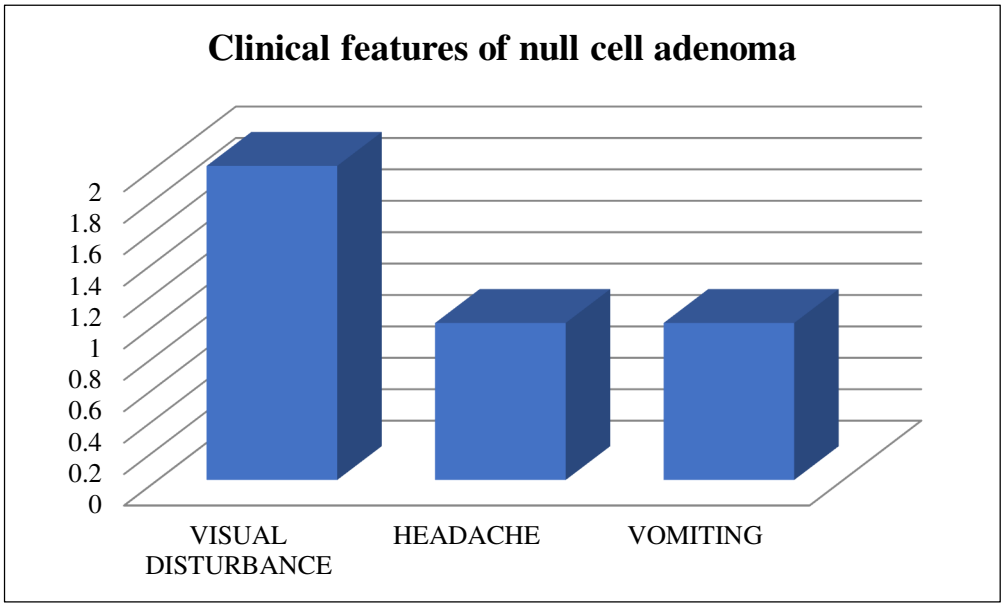


Figure 16: Clinical features of null cell adenoma

All four patients presented with pressure symptoms like visual disturbance (3 cases) followed by headache (1 case) and vomiting (1 case).

Thyrotroph adenoma:

None of the 50 cases examined showed cytoplasmic positivity for TSH IHC.

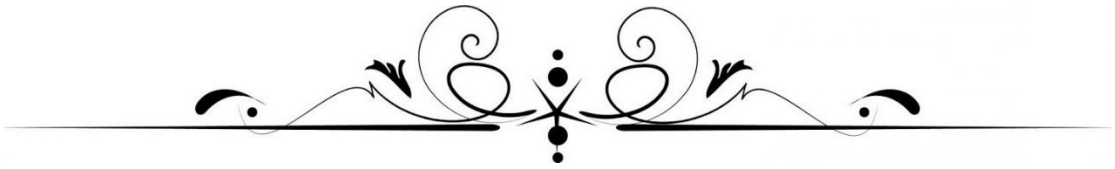
The salient features are summarized in the following table

Group	Features	Frequency (total number of cases=50)	Percentage (%)
Age (in years)	≤30	7	14
	31-50	32	64
	>50	11	22
Gender	Male	35	70
	Female	15	30
Size	Macroadenoma	39	78
	Giant cell adenoma	10	20
	Microadenoma	1	2
Ki-67 Li	<3%	44	88
	≥3%	6	12
Clinical features	Headache	29	58
	Visual disturbance	26	52
	Acromegaly	12	24
	Nausea, vomiting	9	18
	Cushing's disease	1	2
	Trigeminal neuralgia	1	2
	Loss of consciousness	1	2
	Seizure	1	2
	Weight gain	1	2
	Weakness	1	2
Apoplexy	Present	6	12
Clinical type	Non-functional	32	64
	Functional	14	28

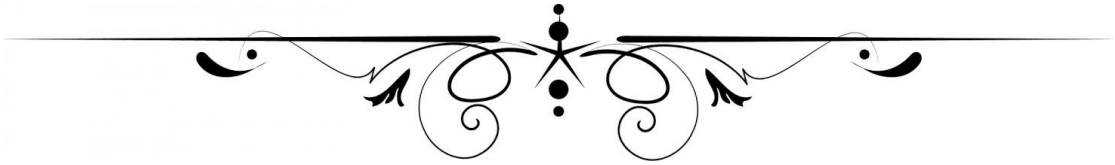
Histological type	Gonadotroph adenoma	22	44
	Somatotroph adenoma	15	30
	Corticotroph adenoma	4	8
	Mammosomatotroph adenoma	1	2
	Lactotroph	3	6
	Plurihormonal and double adenoma	1	2
	Null cell adenoma	4	8
Recurrence	Non-recurrent	44	88
	Recurrent	6	12

Table 13: Summary of observation and results

The above table shows that most of the patients were in their 3rd and 4th decades of life. The majority of the patients were male. According to size, macroadenoma were the most common type of PA. Most of the patients had Ki-67 LI <3%. Clinically, Non-functional adenomas were the most common type and the most common symptoms were headache and visual disturbance. 6 cases showed apoplectic changes. Immunohistologically, gonadotroph adenoma was the most common type and recurrence was uncommon.



DISCUSSION



DISCUSSION

Pituitary adenoma (PA) is the most common benign neoplasm of the pituitary gland and constitutes approximately 15% of all intracranial neoplasms. The correct classification of these tumors is challenging and requires the assembly of clinical details, radiological findings, morphological characteristics and immunohistochemistry. PA can be classified into various categories like functioning and non-functioning based on their hormone secreting capabilities, on basis of size as microadenoma, macroadenoma or giant adenoma and morphologically according to their tinctorial properties and immunohistochemically according to their hormonal contents. Immunohistochemistry is the gold standard for the diagnosis of PA and their subclassification into seven main subtypes as per WHO (4^{th} edition, 2017) [(7)]: Somatotroph adenoma, lactotroph adenoma, thyrotroph adenoma, corticotroph adenoma, gonadotroph adenoma, null cell adenoma and plurihormonal adenoma. The WHO bluebook (4^{th} edition, 2017) advocates the routine use of IHCs for GH, PRL, β-TSH, ACTH, β-FSH, β-LH and α-subunit of glycoprotein**.** It also recommends the use of IHC for transcription factors: Pit-1, SF-1 and Tpit.

This is an observational ambispective, hospital-based observational study conducted on 50 cases of PA at the All India Institute of Medical Sciences (AIIMS), Jodhpur in the Department of Pathology & Lab Medicine.

In this study, the expression of Pit-1 and SF-1 along with Ki-67 LI and IHCs for hormonal markers were studied and the adenomas were classified as per the WHO (4^{th} edition, 2017).

Age distribution of pituitary adenoma cases:

The present study showed 39 cases (78%) in ≤50 years age group, and 11 cases (22%) in >50 years age group. The mean age was 42.62 years and the median age was 42 years, with an age range of 18-77 years. Most of the patients were in their 3^{rd} and 4^{th} decades.

According to Surveillance, Epidemiology, and End Results (SEER) database from the United States, 73% of the patients with PA presented between 19-64 years and the mean age at diagnosis was 49.4 years [(33)]. PA rarely occurs in the pediatric population. There was no patient below 18 years our study. Studies on the Indian population by Rishi A et al found the mean age to be 46.2 years and Chacko et al found the median age to be 40 years [(17,34)].

Gender distribution of PA cases:

In literature, the gender predominance among PA cases is variable. The present study showed a male to female ratio of 2.3:1. According to the SEER database from the United States, the male to female ratio was 1:1.3 [(33)]. Indian studies showed an increased prevalence amongst males. Rishi et al observed a male to female ratio of 2.1:1 [(34)] and study by Chacko et al showed a male to female ratio of 1.4:1 [(17)].

Size wise distribution of PA cases:

The most common type of adenoma according to size distribution in the present study were macroadenomas, comprising of 39 cases (78%), followed by giant adenomas, 10 cases (20%) and 1 case (2%) of microadenoma.

Population based and autopsy studies show an increased incidence of pituitary microadenomas. These incidental findings do not reflect in the clinical settings where patients with microadenomas, restricted to the sella turcica with no endocrinal or pressure symptoms usually do not undergo screening tests. Most of the patients have non functioning adenomas and present to the clinician after the adenomas had grown in size. The usual presentations are due to mass effect (like visual disturbances, headache, nausea and vomiting), due to pressure symptoms on the optic chiasma, pituitary stalk, cavernous sinus invasion or suprasellar and sphenoid sinus invasion.

The classification of PA by size is still widely used, but it has little impact on decision making, and size does not always accurately predict the symptoms.

Clinical presentation:

PAs have a clinical spectrum ranging from “functioning adenoma” to “whispering” to “completely silent” adenoma. The term “silent pituitary adenoma” (SPA) is reserved for those PAs that express one or more anterior pituitary hormones or transcription factors but do not secrete hormones at clinically relevant levels. Thus, if the clinical findings and pathological findings are combined, the clinical diagnosis of NFPA could interchangeably be used with SPA. Clinically NFPAs are typically macroadenomas, and patients present with mass effect [(35)]. Headache and vomiting are caused due to dural irritation, visual disturbances like loss of vision and hemianopias are due to compression of the optic chiasma. In our study, we found 14 cases (28%) of functioning pituitary adenomas and 32 cases (64%) of clinically

non-functioning pituitary adenomas. Clinical details of 4 patients were not available. Clinically NFPA patients presents most commonly presented with headache (58%) followed by visual disturbance (52%) and vomiting (18%). Other features like meningitis, osteoporosis, weakness, weight gain, loss of consciousness and trigeminal neuralgia were also seen in 1 patient each.

A significant fraction of the PA present as incidentalomas and correct subtyping of pituitary adenomas by their hormonal characteristics is necessary for formulating an optimal treatment course for the patient. The treatment strategies for PA include [36] :

1. Suppression of excess hormone secretion and reversal of its clinical consequence
2. Reduction of tumor size and relief of mass effects
3. Preservation of remaining pituitary function
4. Prevention of disease progression and recurrence

In our study, we analyzed the expression for the hormonal IHCs of the anterior pituitary namely GH, PRL, ACTH, β-TSH, β-LH and β-FSH. We also used transcription factors like Pit-1 and SF-1 which are required for further establishing pituitary lineage. α-SU was not used as it was not available at the time of commencing our study and also because it can show positivity for many types of adenomas and may have created diagnostic confusion. Tpit was not used and it was substituted with ACTH IHC, as was previously done by McDonald et al.[23]

In our study, the commonest PA was gonadotroph adenoma (44%), followed by somatotroph adenoma (30%), Corticotroph adenoma (8%), Lactotroph adenoma (6%), Null cell adenoma (8%), Plurihormonal adenomas (2%) and mammosomatotroph adenoma (2%). We did not find any thyrotroph adenoma in our series.

Study	Demographics	Collection period	Total PA cases	Prevalence/ 1 lakh	Proportion of all PAs (%)					
					PRL	ACTH	GH	NFPA	Macro	Micro
Agustsson et al[37]	Iceland	58 years (1955-2012)	471 (M-190, F-281)	116	39.9	11.3	5.7	43.1	54.8	41.2
Gruppetta et al[38]	Malta	11 years (2000-2011)	316 (M-96, F-220)	76	46.2	2.2	16.5	34.2	43.4	56.6
Daly et al[39]	Belgium	14 years (1991-2005)	68 (M-22, F-46)	94	66.2	5.9	13.2	14.7	42.6	57.3
Fernandez et al[40]	United kingdom	1 year (2006)	63 (M-21, F-42)	78	57	2	11	28	41.3	58.7
Raappana et al[41]	Finland	17 years (1992-2007)	164 (M-47, F-117)	68	51	3	8.5	37	54	46

Table 14: Summary of population-based studies showing the prevalence of pituitary adenoma [36]

Gonadotroph adenoma:

In our study, the most common adenoma was gonadotroph adenoma and comprised 22 (44%) of total cases. According to literature, 25-29% of surgically removed tumors were diagnosed as clinically non-functioning adenoma and 43-64% were classified as GA[42–44]. The incidence and prevalence rates of these tumors are difficult to estimate and vary in literature. They occur in middle-aged and older people and have slight male preponderance. They are

usually rare among individuals <25 years. Epidemiologically, our findings are strongly correlating with that mentioned in literature. Our cases comprised of 18 male patients and 4 female patients and the mean age was 45.64 years spanning through 27 - 77 years. They usually occur as macroadenomas and we found 16 macroadenomas, 5 giant cell adenomas and 1 microadenoma. Most gonadotroph adenomas are clinically non functioning and have normal pubertal development and fertility. Our cases presented with mass effects like visual disturbance, headache, vomiting, trigeminal neuralgia and loss of consciousness. Biochemically, the serum markers were predominantly normal. However, 1 case had concomitant TSH elevation. This patient's TSH IHC was reviewed and was found to be negative. With the widespread use of CT and MRI, the number of patients with incidentally detected gonadotrophic adenomas is increasing. Since surgery is the main treatment of choice for these adenomas, this high incidence in surgical series is justified. Histologically, they are comprised of chromophobic adenomas with variable PAS positivity. A distinctive perivascular papillary pseudorosette arrangement is a prominent and consistent histological finding. The individual cells are small to medium-sized and polygonal in shape. In literature, the most common immunohistological subtype is the β-FSH subtype followed by the β-LH subtype, but our findings differed. We found 14 cases to be positive for β-LH and 10 cases amongst them showed additional positivity for β-FSH. Most GA show variable IHC expression and the proportion of immunopositive cells varies from one adenoma to other and from one area to other in the same tumor. These variations may be due to cellular heterogeneity or may be due to differences in fixation, in the specificity of antibodies, antigen retrieval, or signal amplification techniques. Automated platforms and manual techniques also give variable results [(45)].

Since we applied all the gonadotrophic IHCs manually without any additional amplification step, 8 of the 11 (72.7%) hormone negative adenomas which showed strong nuclear positivity for SF-1 were recategorized into silent gonadotrophin adenomas in our study. 10 of the patients had normal serum biochemical levels and 1 patient had hyperthyroidism. These tumors did not show any additional immunoreactivity for other hormonal markers or Pit-1 transcription factor. Thus, these cases were included as gonadotroph adenomas. Corroborating with the findings of other authors who successfully classified clinically non-functioning adenomas with IHC and ancillary techniques like rRNA sequencing, our addition of 8 cases in the category of GA is justifiable.

This finding is in concordance with previously published literature which suggests that many hormone negative adenomas are indeed SF-1 positive GA [46]. Hiroshi et al. found 79 (66.4%) of 119 hormone negative adenomas to be positive for SF-1 [43].
Recurrence is common after surgical removal. The Ki-67 LI is the most reliable predictor of recurrence in gonadotroph adenoma [7].

Somatotroph adenoma:

Somatotroph adenoma (SA) is the second most prevalent type of PA in our study. These tumors account for approximately 10-15% of all resected PA and the mean patient age at diagnosis is 47 years with an approximately equal sex distribution [7,42]. The mean age in our study was 41.33 years and the gender ratio was almost equal (8 males and 7 females). 11 of the 50 cases were macroadenomas and 4 cases were giant adenoma. Acromegaly and pituitary gigantism are known to be associated with pituitary somatotroph adenomas. 10 (66.7%) of our patients had features of acromegaly. The others had pressure symptoms like headache, visual disturbance, vomiting and seizures.

Preoperative clinical diagnosis of SA is done by measuring increased levels of serum GH and insulin-like growth factor 1 (IGF-1). Receptors for GH are predominantly found on the liver and cartilage. GH binds to these receptors and stimulates the liver to produce IGF-1. Our study showed 13 out of 15 patients had increased serum GH/IGF-1 levels. Additionally, one patient also had increased serum prolactin levels but this can be due to the "Pituitary stalk effect" [47].

Microscopically few of these SA showed presence of fibrous bodies, which are juxtanuclear keratin aggresomes. These fibrous bodies showed dot-like peri nuclear positivity on cytokeratin IHC. All these SA showed diffuse strong expression of GH IHC and all the 15 cases showed positive expression for Pit-1 IHC, conforming to the PIT-1 lineage of these adenomas. 2 cases also showed strong nuclear positivity for SF-1 IHC. In literature, rare cases showing positivity for SF-1 in SAs are mentioned, our findings could have been more defined and streamlined if we could have used multiple clones of SF-1 and compared the results.

Overall, we found IHC for GH and Pit-1 to be a very sensitive and specific marker for detecting somatotroph adenoma.

Corticotroph adenoma:

In our study, we found 4 cases (8%) of corticotroph adenoma (CA). According to literature, they account for approximately 15% of all PA with a male predominance and peak incidence within 30-50 years [(40)]. Our study showed equal gender prevalence (2 males and 2 females), the mean age of 41.25 years and the age range was 35-53 years. Most corticotroph adenomas are biochemically functioning. However, 20% lack the clinical and biochemical evidence of ACTH or cortisol excess. These tumors are described as "silent corticotroph adenoma (SCA)" and were first reported by Kovacs et al. as a distinct clinicopathological entity [(17)]. Biochemical profiles of SCA differs from functioning corticotroph adenomas and usually are immunoreactive for ACTH IHC. Individual corticotroph cells in SCA most probably secrete insufficient, inactive ACTH molecules and they are proposed to arise from dysregulated processing of POMC-producing cells in the pars intermedia and not from the anterior pituitary gland [(48,49)]. These are detected incidentally or when the tumor impinges on surrounding structures.

1 of the 4 patients had Cushing's disease with elevated cortisol levels. Three patients presented with pressure symptoms, one of whom had galactorrhoea and elevated prolactin levels. One patient also had hypothyroidism.

Histologically, these cells were basophilic and showed positive PAS reactivity. On Immunohistochemistry, these cells showed diffuse cytoplasmic positivity for ACTH IHC and showed ring-like cytoplasmic positivity on CAM 5.2 IHC.

Rarely, some SCA lack ACTH expression but express TPIT. It is possible that we missed out on some SCA by not using Tpit IHC. Currently, IHC for Tpit is not widely available in general practice and this lineage is usually identified by ACTH immunoreactivity as we have followed in our study. Nishioka et al found that amongst 119 hormone negative adenomas with <1% entrapped ACTH positive cells, 32 cases were immunopositive for Tpit [(43)].

In literature, it has been seen that SCA is a pathologic and clinical distinct entity and few corticotroph adenomas also show positivity for SF-1 and are seen to exhibit features and differentiation common to both corticotroph and gonadotroph lineages. Pulichimo et al proposed that antagonism between Tpit and SF-1 may play a role in the early establishment of POMC and gonadotroph lineages and that these lineages may arise from common precursors [(50)]. Suzuki M et al also found 5 corticotroph adenomas to co-express Tpit and SF-1 [(51)]. Cooper O et al found 80% of functional CA and 100% SCA to be positive for SF-1 and

negative for Tpit. They found both nuclear and cytoplasmic positivity of SF-1 in SCA cases[52].

McDonald et al did not initially use Tpit IHC in one of their studies, but later followed up their study with Tpit IHC and reclassified 8 of 18 (44%) PAs as SCA [53].

Lactotroph adenoma:

In our study, only 3 (6%) of the 50 cases were pure lactotroph adenoma. According to literature, they are the most common type, accounting for 30-50% of pituitary adenomas in autopsy and incidental prevalence series but their incidence is very low in surgical series due to successful pharmacotherapy [39,46,54]. Indeed, these are the only intracranial neoplasm that can be treated medically. Usually, lactotroph adenomas have a female predilection and occur more commonly in adults aged 21-40 years. We found 2 cases in males and 1 female. The patients were between 21-50 years and both male patients presented with increased serum prolactin levels. In our study, all three cases were macroadenomas. The female patient presented with pressure symptoms of visual disturbance. She did not have other features like galactorrhoea/ovulatory disorder/delayed puberty/menstrual irregularities. In a randomized, double-blind study of 459 women with hyperprolactinemia, 83 percent of those treated with cabergoline achieved normoprolactinemia[36,55]. The use of these drugs makes the cells dramatically smaller, with cytoplasmic shrinkage and increased nucleo-cytoplasmic ratio along with tumor fibrosis. These treatment effects also have variability on the expression of PRL IHC. In a resource-limited demographic area like Rajasthan, where more than 75% of people are rural dwellers [56], past medical reports are not usually digitalized, and patients, mostly women, are not usually aware of their treatment history which may be a contributing factor to less LA cases in our study.

Microscopically, the tumors were arranged in diffuse sheets and sinusoidal patterns. The cells were predominantly monomorphic, round to oval with eosinophilic to chromophobic cytoplasm. In our study, one lactotroph adenoma was noted with co-existing meningioma. This LA had intranuclear pseudo inclusions. Such inclusions are reported in the literature and are found to be more common in prolactinomas, similar to our case [57]. IHC for PRL showed diffuse and strong cytoplasmic expression in all our cases. Nuclear expression for Pit-1 IHC was strong thus, confirming the lineage of these adenomas. However, 2 cases concomitantly showed strong nuclear expression for SF-1 as well.

Mammosomatotroph adenoma:

In our study, 1 case was found to be immunopositive for both GH and PRL and was classified as mammosomatotroph adenoma. This 18-year male patient had elevated serum levels for both growth hormone and prolactin. This tumor showed predominantly eosinophilic cytoplasm and exhibited GH and PRL IHC positivity in the same group of cells. Mammosomatotroph adenomas are very frequently seen in young adults with acromegaly [58]. Our patient had macroadenoma and presented with features of acromegaly and had pressure symptoms like headache.

Plurihormonal adenoma:

The incidence of plurihormonal Pit-1 adenoma is 0.9% and that of double adenomas is 0.4% - 1.3% in surgical series [59]. In our study, 1 case (2%) was immunopositive for GH-ACTH and this was categorized as plurihormonal adenoma. This 39 years male patient had increased serum IGF-1 level and testosterone level. Clinically, he had a macroadenoma and features of acromegaly along with pressure symptoms like visual disturbance and headache. This case showed immunopositivity for both Pit-1 and SF-1.

The Ki-67 LI of the case was 2%.

Thyrotroph adenoma:

We did not find any thyrotroph adenoma in our study. A few of the cases which showed positive expression for TSH were meticulously studied and correlated with serum thyroid profile, clinical history, H & E sections, reticulin staining and other hormonal and transcriptional factor's expression. We found those cells to be entrapped normal pituitary cells. Our findings are corroborating with that of literature which mentions thyrotroph adenoma to be a rare tumor and accounts for <2% of all PAs [60,61].

Null cell adenoma:

In our study, 4 cases (8%) were immunonegative for all hormonal IHC markers and transcription factors SF-1 and Pit-1. As per literature, hormone-immunonegative markers account for 5-30% and both hormone and transcription negative adenomas account for <5% of all surgically removed, clinically non-functioning adenohypophyseal tumors [43,62,63]. They are usually found in elderly individuals and have a slight male preponderance. All 4 patients in our study were male and the mean age was 43.75 years. All 4 cases had macroadenomas

and presented with mass symptoms like visual disturbance, headache and vomiting. Their biochemical serum markers were within normal limits. Histologically, the cells were round to oval with chromophobic to weakly acidophilic cytoplasm and arranged in diffuse sheets. No perivascular pseudorosetting, fibrous bodies or hyaline globules were noted. Hence, these were excluded from SGA/SCA. Null cell adenoma is predominantly a diagnosis of exclusion and herein lies the significance of using all the 3 transcriptions factors which are available to date. As mentioned earlier, variability in staining property is dependent on various factors and since we did not use IHC for Tpit, one or a few silent corticotroph adenomas could have been missed.

Nishioka et al studied transcription factors on 516 NFPA and found 5% of the cases to be null cell adenoma [(43)].

Surgery is the treatment of choice for null cell adenomas and their prognosis is favorable [(64)].

Ki-67 LI:

Ki-67 LI is used as a proliferative marker in PA. Previously, tumors having a high mitotic index, Ki-67 proliferation index of >3% and strong nuclear p53 staining were classified as "atypical adenoma". The WHO, (4th edition, 2017) has removed the term "atypical adenoma" but the assessment of tumor proliferation by Ki-67 is still recommended. Ki-67 values aid in predicting the recurrence after surgery.

In our study, we found the highest mean Ki-67 LI in LA (1.66%), followed by GA (1.53%) and the least mean Ki-67 LI was seen in SA (1.4%). There was only a single case of mammosomatotroph adenoma which showed 3% Ki-67 LI. The range varied from 0% to 4% and the overall mean value of all the PA was 1.46%. Chacko et al found a mean value of 1.26% and they found no correlation between either tumor volume or maximum tumor diameter and Ki-67 LI. (17) A study by Sen A et al showed only 4 (13%) out of 32 patients had >3% Ki-67 LI. Knosp et al found the range of Ki-67 Li to lie within 0.1- 2.8% in the 62 cases they studied. Rishi et al found 58 of 77 NFPA cases to have Ki-67 less than 1% [(34)].

Pit-1:

The role of Pit-1 and its correlation with the expression of GH and PRL in our study is in concordance with previously published literature. This member of the POU-domain family is a transcription factor involved in the normal development of the pituitary. Although Pit-1 mRNA transcripts are present in all pituitary cells, it is only the tumorous cells that secrete

Pit-1 protein leading to their detection by IHC. We found all our 17 SA and 3 LA to be positive for Pit-1. None of the GA or CA were positive for Pit-1. Thus, Pit-1 showed excellent sensitivity and specificity in our study.

SF-1:

SF-1 is predominantly expressed in gonadotroph adenoma and has been extensively used to classify hormone negative adenomas to silent gonadotroph adenoma and null cell adenoma. The routine use of β-FSH and β-LH can also be replaced with SF-1, which will decrease the total number of IHCs used in the PA panel as has been suggested by McDonald et al. [(23)].

Our findings showed excellent sensitivity of SF-1 for detecting gonadotroph adenomas but low specificity as it showed strong positivity in 1 ACTH positive, 1 GH-ACTH positive, 2 PRL positive and 2 exclusively GH secreting cases as well. Thus, our classification of 8 cases into SGA leaves room for error of misclassification. The sensitivity and specificity of SF-1 are also debatable in literature and until a very accurate IHC is available, we cannot confidently classify all the SF-1 positive tumors as SGA. A recent molecular classification that analyzed PA by integrated pangenomics found gonadotroph signature in some CA and SA as well, thus raising a challenge to the current SF-1 lineage and the specificity of this marker [(65)]. In a cohort of 30 patients, JVG Tamanini et al showed SF-1 expression along with Pit-1 and Tpit in patients with acromegaly, Cushing's disease [(29)].

Tpit :

We did not use T-pit which aids in differentiating corticotroph adenomas from other adenomas. Most corticotroph adenomas are ACTH positive and we substituted Tpit with ACTH.

Limitations of the study

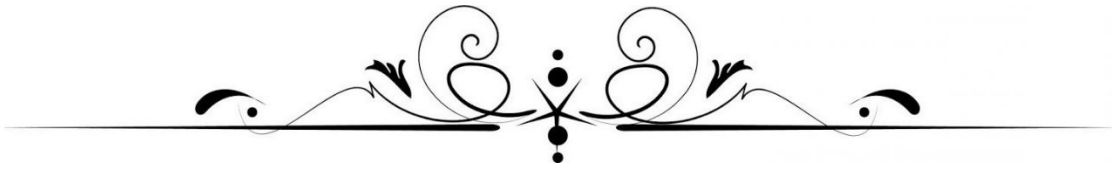
- We did not use IHC for α-SU and Tpit. We may have missed a few of the silent corticotroph adenoma by not using Tpit.
- Complete clinico-radiological details were not available for few of the patients.
- SF-1 showed positivity in a wide range of tumors.

Strengths of the study

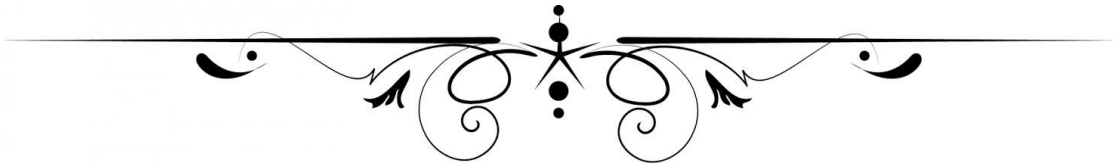
- We included a substantial number of 50 cases in our study.
- Extensive workup was done for all the cases. We correlated clinical details, radiological findings, morphological characteristics and immunohistochemistry to reach a conclusion about each and every case.
- The strength of our study depends on the simultaneous use of all the hormonal IHC markers (except for α-SU) and two (Pit-1 and SF-1) of the three most widely used transcription factors (Pit-1, SF-1 and Tpit). We also used Ki-67 LI for assessing the proliferative activity of the tumors.
- A vast majority of cases which were clinically non functioning adenoma were successfully classified according to WHO, 4e(2017).
- Classifying the cases into definite subtype has therapeutic implications as newer drug therapies are available against specific molecular targets. Everolimus, a mTOR inhibitor and lapatinib, a tyrosine kinase inhibitor for EGFR has shown efficacy in aggressive prolactinomas. Oral SST2 receptor agonist like Paltusotine is an emerging drug for treatment of acromegaly. Several novel pituitary-directed drugs like Roscovite, a CDK inhibitor are in development for the treatment of Cushing's disease.

Recommendations for further work

- Complete follow up of patients with study of survival analysis and observation of recurrence rates.
- IHC for Tpit and other clones of SF-1 will be used on the existing cases and the results will be corroborated further.



SUMMARY & CONCLUSIONS



SUMMARY AND CONCLUSIONS

Expression of Pit-1, SF-1 and Ki-67 LI was studied along with the hormonal IHC markers of anterior pituitary in 50 cases.

- Most of the patients were in their third and fourth decades. No pituitary adenoma was found in pediatric patients in our cases.
- GH was positive in 17 cases of which 15 were somatotroph adenomas, 1 was mammosomatotroph adenoma and 1 was plurihormonal adenoma.
- PRL was positive in 5 cases, of which 3 were lactotroph adenomas, 1 was mammosomatotroph adenoma and 1 was plurihormonal adenoma.
- β-TSH was not positive in any of the cases.
- β-LH was positive in 14 gonadotroph adenomas. β-FSH and β-LH both were positive in 10 gonadotroph adenomas.
- ACTH was positive in 6 cases of which 4 were corticotroph adenomas and 2 were plurihormonal adenomas.
- All the hormonal markers done were entirely negative in 12 of 50 cases. However, 8 of these cases could be further categorized as gonadotroph adenomas due to SF-1 expression and negativity for Pit-1.
- Pit-1 was positive in 20 cases. 15 of these being gonadotroph adenomas, 3 were lactotroph adenomas, 1 mammosomatotroph and 1 was plurihormonal adenoma.
- SF-1 was positive in 28 cases of which 14 were gonadotroph adenomas, 2 were somatotroph adenomas, 2 lactotroph adenomas, 1 plurihormonal adenoma, 1 corticotroph adenoma and 8 were hormone-negative adenomas.
- Ki-67 LI ranged from 0%-4%.

Thus, we were able to classify the 50 PAs into Gonadotroph adenoma (44%), Somatotroph adenoma (30%), Corticotroph adenoma (8%), Lactotroph adenoma (6%), Null cell adenoma (8%), Plurihormonal adenoma (2%) and Mammosomatotroph adenoma (2%).

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ANNEXURES

ETHICAL JUSTIFICATION

- Informed written consent will be taken from all the study subjects.
- No pressure or coercion will be exerted on subjects for participation in study.
- Confidentiality and privacy will be maintained at all stages.
- Enrolment in the study will not pose any additional risk to the patient and will not increase the cost of the treatment. Informed written consent will be taken from patient/guardian of all the patients as per the attached proforma.

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/2054 Date: 01/01/2020

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/966**

Project title: **"Expression of Pit-1, SF-1, Ki-67 labelling index in correlation with immunohistochemical expression of hormonal markers in pituitary adenomas"**

Nature of Project:	**Research Project**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr.Ismetara Begam**
Guide:	**Dr.Poonam Abhay Elhence**
Co-Guide:	**Dr.Sudeep Khera, Dr. Deepak Vedant, Dr.Madhukar Mittal & Dr. Mayank Garg**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **23-12-2019** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure 1

Basni Phase-2, Jodhpur, Rajasthan-342005, **Website:** www.aiimsjodhpur.edu.in, **Phone:** 0291-2740741 Extn. 3109
Email: ethicscommittee@aiimsjodhpur.edu.in

Annexure I

Institutional Ethics Committee
All India Institution of Medical Sciences, Jodhpur

Meeting of Institutional Ethics committee held on **23-12-2019 at 10:00** AM at Committee Room, Admin Block AIIMS Jodhpur.

Following members were participated in the meeting:-

S/No.	Name of Member	Qualification	Role/Designation in Ethics Committee
1.	Dr. F.S.K Barar	MBBS, MD (Pharmacology)	**Chairman**
2.	Justice N.N Mathur	LLB	Legal Expert
3.	Dr. Varsha Sharma	M.A (Sociology)	Social Scientist
4.	Mr. B.S.Yadav	B.Sc., M.Sc. (Physics), B.Ed.	Lay Person
5.	Dr. K.R.Haldiya	MD (General Medicine)	Clinician
6.	Dr. Arvind Mathur	MBBS, MS (General Medicine)	Clinician
7.	Dr. Surajit Ghatak	MBBS, MS (Anatomy)	Basic Medical Scientist
8.	Dr. Vijaya Lakshmi Nag	MBBS, MD (Microbiology)	Basic Medical Scientist
9.	Dr. Sneha Ambwani	MBBS, MD (Pharmacology)	Basic Medical Scientist
10.	Dr. Kuldeep Singh	MBBS, MD (Paediatric), DM (General Medicine)	Clinician
11.	Dr. Abhinav Dixit	MBBS, MD (Physiology), DNB (Physiology)	Basic Medical Scientist
12.	Dr. Pradeep Kumar Bhatia	MBBS, MD (Anaesthesiology)	Clinician
13.	Dr. Tanuj Kanchan	MBBS, MD (Forensic Medicine)	Basic Medical Scientist
14.	Dr. Pankaj Bhardwaj	MBBS, MD (CM&FM)	Clinician
15.	Dr. Praveen Sharma	M.Sc., Ph.D. (Biochemistry)	Member Secretary

Dr. Praveen Sharma
Member Secretary
Institutional Ethics Committee
AIIMS Jodhpur

All India Institute of Medical Sciences

Jodhpur, Rajasthan

Informed Consent Form

Title of the project : Expression of Pit-1, SF-1, Ki-67 labelling index in correlation with IHC expression of hormonal markers in pituitary adenomas

Name of the Principal Investigator : Dr. Ismetara Begam Tel. No. 8981203356

Patient/Volunteer Identification No. :_____

I, _____ S/o or D/o _____ R/o _____give my full, free, voluntary consent to be a part of the study "_____-_____", the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individual from _____ -(Company Name) or from regulatory authorities. I give permission for these individuals to have access to my records.

Date : _____ _____

Place : _____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date : _____ _____

Place : _____ Signature of Principal Investigator

Witness 1

Signature _____

Name: _____

Address : _____

2. Witness 2

Signature _____

Name: _____

Address : _____

All India Institute of Medical Sciences

Jodhpur, Rajasthan

informed consent form(Hindi)

थीसिस / निबंधकाशीर्षक: **एक्सप्रेशन ऑफ़** Pit -1 , SF-1 , Ki-67 **लाबेल्लिंग इंडेक्स इन करेलशन विथ इम्मुनोहिस्तोचेमिकल एक्सप्रेशन ऑफ़ हार्मोनल मार्कर्स इन पिट्यूटरी अडेनोमास**

पीजी छात्र का नाम: डॉ. इसमेतारा बेगम **टेलन**: 8981203356

रोगी / स्वयंसेवक पहचान संख्या: _____

मैं, _____**एस / ओयाडी / ओ** _____**आर/ओ**_____ _____**अध्ययन** "**एक्सप्रेशन ऑफ़** Pit -1 , SF-1 , Ki-67 **लाबेल्लिंग इंडेक्स इन करेलशन विथ इम्मुनोहिस्तोचेमिकल एक्सप्रेशन ऑफ़ हार्मोनल मार्कर्स इन पिट्यूटरी अडेनोमास**" **का एक भाग बनने के लिए मेरी पूर्ण**, **स्वतंत्र**, **स्वैच्छिक सहमति दें**,**जिसकी प्रक्रिया और प्रकृति मुझे अपनी पूरी संतुष्टि के लिए अपनी भाषा में समझाई गई है। मैं पुष्टिकरता हूं कि मुझे प्रश्न पूछने का अवसर मिला है।**

मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मुझे किसीभी कारण दिए बिना किसीभी समय अध्ययन से बाहर निकलने के मेरे अधिकार की जानकारी है।

मैं समझता हूं कि मेरे और मेरे मेडिकल रिकॉर्ड के बारे में एकत्रित की गई जानकारी को_____(**कंपनीनाम**) **या विनियामकप्राधिकरणों से जिम्मेदार व्यक्ति द्वारा देखा जा सकता है।मैंइ नव्यक्तियोंको अपने अभिलेखोंतक पहुंच के लिए अनुमति देता हूं**I

रीख : _____

जगह: _____ **हस्ताक्षर / बाएं अंगूठे का छाप**_____

यह प्रमाणित करने केलिए कि मेरी उपस्थिति में उपरोक्त सहमति प्राप्त की गई हैI

तारीख : _____

जगह: _____**पीजी छात्र के हस्ताक्षर**_____

गवाह1: _____ हस्ताक्षर: _____ □ारीख : _____	गवाह**2**: _____ हस्ताक्षर: _____ □ारीख : _____

PATIENT INFORMATION SHEET

1. Risks to the patients: No interventions or life-threatening procedure will be done.

2. Confidentiality: Your participation will be kept confidential. Your medical records will be treated with confidentiality and will be revealed only to doctors/ scientists involved in this study. The results of this study may be published in a scientific journal, but you will not be identified by name.

3. Provision of free treatment for research related injury. Not applicable.

4. Compensation of subjects for disability or death resulting from such injury: Not Applicable

5. Freedom of individual to participate and to withdraw from research at any time without penalty or loss of benefits to which the subject would otherwise be entitled.

6. You have complete freedom to participate and to withdraw from research at any time without penalty or loss of benefits to which you would otherwise be entitled.

7. Your participation in the study is optional and voluntary.

8. The copy of the results of the investigations performed will be provided to you for your record.

9. You can withdraw from the project at any time, and this will not affect your subsequent medical treatment or relationship with the treating physician.

10. Any additional expense for the project, other than your regular expenses, will not be charged from you.

रोगी सूचना पत्रक

1. **रोगियों के लिए जोखिम: कोई हस्तक्षेप या जीवन-धमकी प्रक्रिया नहीं की जाएगी।**

2. **गोपनीयता: आपकी भागीदारी को गोपनीय रखा जाएगा। आपके मेडिकल रिकॉर्ड को गोपनीयता के साथ इलाज किया जाएगा और केवल इस अध्ययन में शामिल डॉक्टरों / वैज्ञानिकों को पता चलेगा। इस अध्ययन के परिणाम एक वैज्ञानिक पत्रिका में प्रकाशित हो सकते हैं, लेकिन आपको नाम से पहचाना नहीं जाएगा।**

3. **अनुसंधान संबंधी चोट के लिए नि: शुल्क उपचार की व्यवस्था। लागू नहीं।**

4. **ऐसी चोट से उत्पन्न विकलांगता या मृत्यु के लिए विषयों का मुआवजा: लागू नहीं है**

5. **किसी भी समय दंड या लाभों के नुकसान के बिना किसी भी समय भाग लेने के लिए व्यक्ति को स्वतंत्रता लेने और अनुसंधान से वापस लेने के लिए स्वतंत्रता, जिसके तहत विषय अन्यथा हकदार होगा**

6. **आपको जुर्माना या लाभ के नुकसान के बिना किसी भी समय भाग लेने और अनुसंधान से वापस लेने की पूरी आजादी है, जिस पर आप अन्यथा हकदार होंगे।**

7. **अध्ययन में आपकी भागीदारी वैकल्पिक और स्वैच्छिक है।**

8. **प्रदर्शन की जांच की परिणामों की प्रति आपके रिकॉर्ड के लिए आपको उपलब्ध कराई जाएगी।**

9. **आप किसी भी समय परियोजना से वापस ले सकते हैं, और यह आपके बाद के चिकित्सा उपचार या उपचार चिकित्सक के साथ संबंध को प्रभावित नहीं करेगा।**

10. **परियोजना के लिए कोई भी अतिरिक्त व्यय, आपके नियमित खर्चों के अलावा, आपसे शुल्क नहीं लिया जाएगा।**

All India Institute of Medical Sciences (AIIMS), Jodhpur
Department of Pathology
PROFORMA

Name:	
Age (in years) :	
Sex:	
Hospital No.:	
Address:	
Phone Number:	

1.BASIC INFORMATION OF PATIENT:

HEIGHT: WEIGHT: BMI:

2. CHIEF COMPLAINTS:

Complaints	Yes	No	Duration and remarks
Nausea and Vomiting			
Headache			
Vision Problems			
Change in behaviour, any psychological changes			
Nasal Drainage			
Menstrual irregularity			
Infertility			

Sexual Dysfunction			
Fatigue			
Unexplained weight gain or weight loss			
Dermatotogical complaints:			
Any additional complaint:			

3.ADDICTION

Nature	**Yes**	**No**	**Duration**
Alcohol			
Smoking			
Tobacco			
Others:			

4. CO-MORBIDITIES

Illness	**Yes**	**No**	**Duration**
Systemic Hypertension			
Diabetes Mellitus			
CAD			
Respiratory Problems			
Others			

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NEUROLOGICAL EXAMINATION:

GYNAECOLOGY & OBSTETRICS HISTORY (ALONG WITH MENSTRUAL HISTORY):

RELEVANT FAMILY HISTORY:

HISTORY OF MEDICATIONS:

IMAGING DETAILS:

HISTO-PATHOLOGICAL RESULTS:

Gross examination:

Microscopic examination:

Results of IHC markers:

(1) IHC: Pit-1: Allred score

Positive Cells (%)	**Proportion Score (P)**	**Intensity**	**Intensity Score (I)**
0	0	None	0
<1	1	Weak	1
1-10	2	Intermediate	2
11–33	3	Strong	3
34–66	4		
>/=67	5		
Total Score is = P+I			

(2) IHC: SF-1 : Allred score

Positive Cells (%)	Proportion Score (P)	Intensity	Intensity Score (I)
0	0	None	0
<1	1	Weak	1
1-10	2	Intermediate	2
11–33	3	Strong	3
34–66	4		
>/=67	5		
Total Score is = P+I			

(3) IHC staining for anterior pituitary hormones:

Hormonal IHC marker	Positive	Negative
GH		
PRL		
β-FSH		
β-LH		
β-FSH		
ACTH		

(4) Other IHC markers done:

- Ki-67 (in all cases)
- CAM 5.2
- Pan-CK
- Chromogranin A
- Synaptophysin
- TTF-1
- p53

(5) Other histochemical stains done: Reticulin, PAS (Periodic acid-Schiff stain)