

**SPECTRUM OF PITUITARY NEUROENDOCRINE TUMORS
(PitNETs)
IN A TERTIARY CARE CENTRE:
CLASSIFICATION BASED ON
2022 WORLD HEALTH ORGANIZATION GUIDELINES**

Dr Silvie Thomas

PDCC in Neuropathology Thesis

2024



**SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND
TECHNOLOGY, TRIVANDRUM**

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A THESIS SUBMITTED BY

Dr Silvie Thomas

TO

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND
TECHNOLOGY, TRIVANDRUM.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF

PDCC in Neuropathology

2024

DECLARATION BY THE STUDENT

CERTIFICATE

I, Dr Silvie Thomas, hereby certify that I had personally carried out the work depicted in the thesis titled “***Spectrum of pituitary neuroendocrine tumors (PitNETs) in a tertiary care centre: classification based on 2022 world health organization guidelines***” No part of this thesis has been submitted for the award of any other degree or diploma prior to this date.

Dr. Silvie Thomas,
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Date: 14/02/2025



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The thesis entitled, "*Spectrum of Pituitary Neuroendocrine Tumors (PitNETs) in a Tertiary Care Centre: Classification based on 2022 World Health Organization Guidelines*" was carried out under my direct supervision. No part of the thesis was submitted for the award of any degree or diploma prior to this date.

*Clearance was obtained from the Institutional Ethics Committee of this institute (SCT/IEC/2205/MARCH/2024) for carrying out the study.

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APPROVAL OF THE THESIS

The thesis entitled

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Classification based on 2022 World Health Organization Guidelines*

Submitted by

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PDCC in Neuropathology

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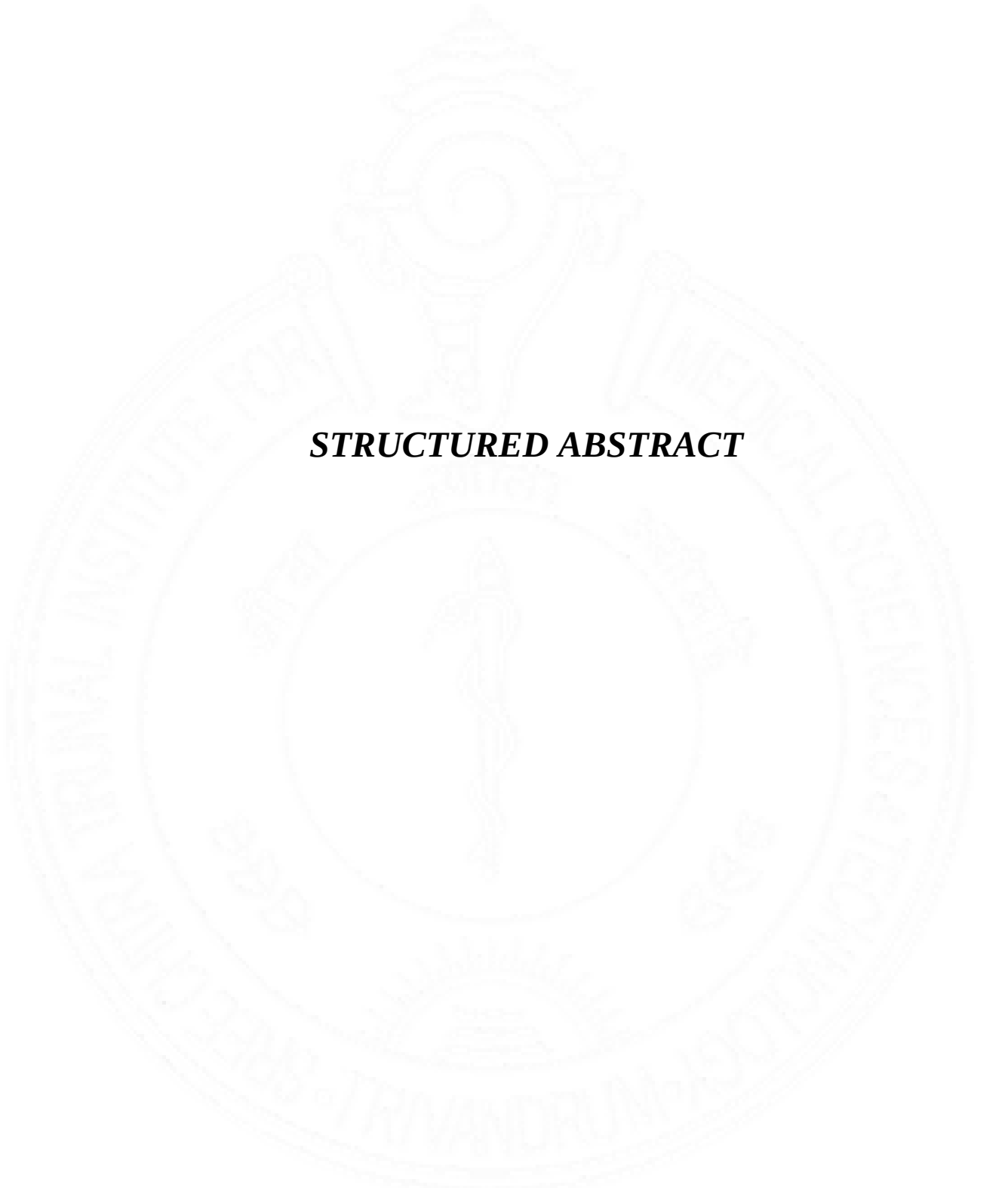
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LIST OF ABBREVIATIONS

Abbreviation	Full form
ACTH	Adrenocorticotrophic Hormone
ER-alpha	Estrogen Receptor alpha
FSH	Follicle stimulating hormone
GH	Growth Hormone
IGF-1	Insulin - like growth factor 1
IHC	Immunohistochemistry
KM	Kaplan-Meier
LH	Luteinizing hormone
MRI	Magnetic Resonance Imaging
mTOR	mammalian target of rapamycin
NENs	Neuroendocrine Neoplasms
NET	Neuroendocrine tumor
PA	Pituitary adenoma
PFS	Progression free survival
PIT1	Pituitary-specific positive transcription factor 1

PitNETs	Pituitary Neuroendocrine Tumours
POMC	Proopiomelanocortin
PRL	Prolactin
SF1	Steroidogenic Factor 1
TMZ	Temozolomide
TPIT	T-box transcription factor
TSH	Thyroid stimulating hormone
VEGF-R	Vascular endothelial growth factor receptors
WHO	World Health Organization



STRUCTURED ABSTRACT

Background

Pituitary adenomas (PA), now termed Pituitary Neuroendocrine Tumours (PitNETs) as per the 2022 WHO classification. This classification of PitNETs relies on evaluating pituitary hormone and transcription factor expression using immunohistochemistry.

Our study aimed to apply the 2022 WHO classification to a large cohort of PitNETs from our Centre with clinico-radiological correlation.

Aims and Objectives

Aim:

To classify PitNETs based on the 2022 WHO classification and to correlate histopathological subtypes with clinico-radiological features.

Objectives:

1. To classify PitNETs by assessing the expression of pituitary hormones and transcription factors characteristic of a specific lineage via immunohistochemistry.
2. To analyze the association between PitNET subtypes and clinico-radiological features.
3. To evaluate the prognostic significance of PitNET subtypes.

Methods:

A retrospective analysis was conducted on 514 PitNETs diagnosed from 2016-2023.

Immunohistochemistry was performed for transcription factors (PIT1, SF1, TPIT, GATA2, ER-alpha), pituitary hormones (GH, PRL, ACTH, TSH-beta, FSH-beta, LH-beta, LH-alpha), Ki-67, p53, and CAM5.2. The PitNETs were classified based on the 2022 WHO classification. The clinical, radiological and follow-up data were collected.

Data analysis was performed with SPSS software (version 30.0.0). The Chi-square test was used to compare categorical variables. Survival analysis was performed using the Kaplan-Meier method and Cox proportional hazards regression. Statistical significance was defined as a p-value of less than 0.05.

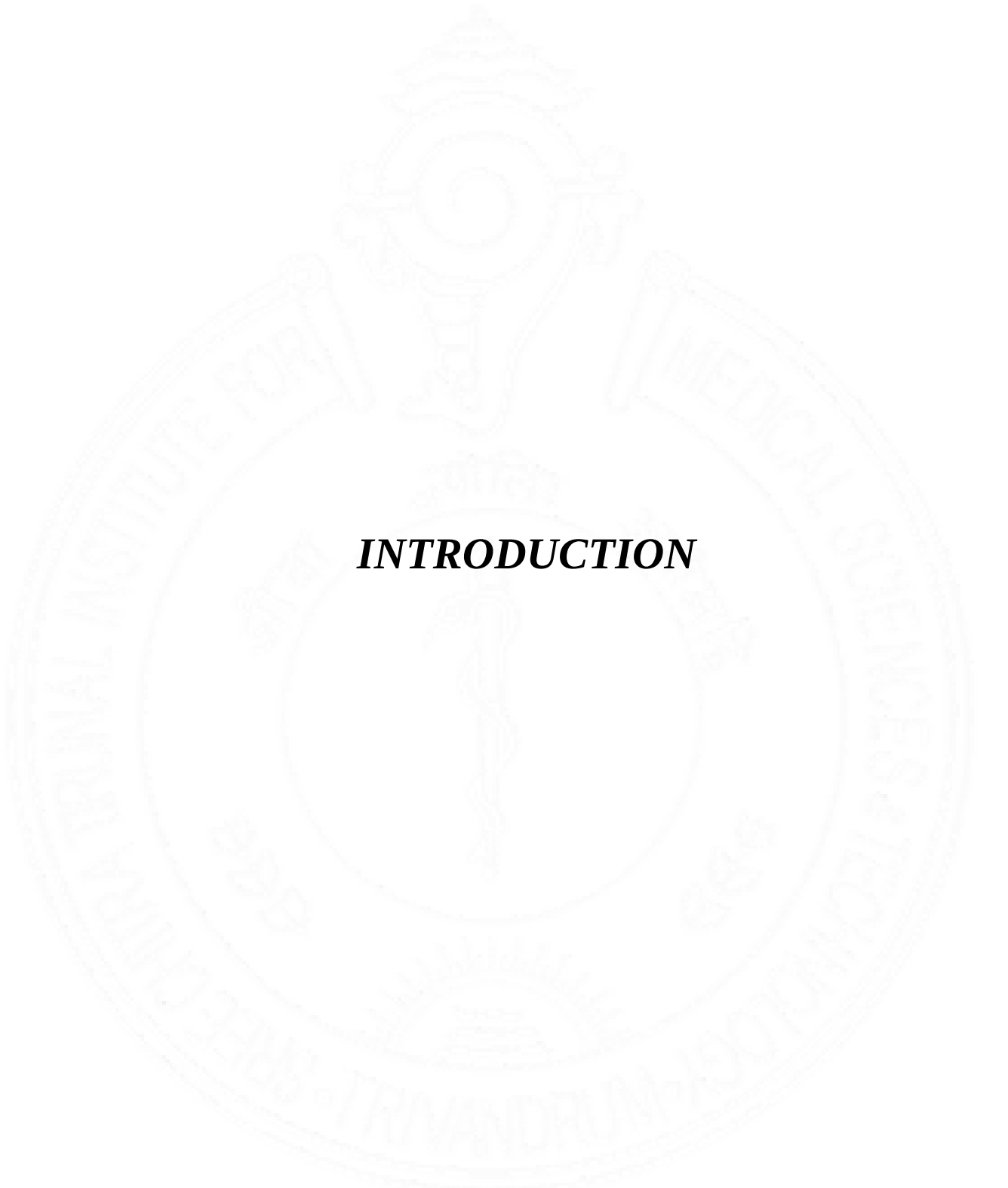
Results:

Among 514 PitNETs, SF1 lineage tumors (41.1%) predominated, followed by TPIT lineage (33.5%). The mean patient age was 46 years. SF1 lineage was more common in males and while TPIT lineage was more common in females. Age distribution varied across lineages, with patients < 30 years more likely to have PIT1 lineage tumors and > 45 years more likely to have SF1 lineage tumors (Chi square $p < 0.001$). The SF1 and TPIT lineages presented mostly with pressure symptoms, while PIT1 lineage tumors had both pressure and endocrine symptoms (Chi square $p < 0.001$). 75% of tumors were macroadenomas and most were SF1 (44.8%) lineage tumors. Invasion was significantly associated with TPIT (43%) lineage tumors (Chi square $p < 0.001$). 63.8% of tumors were Grade 1 and 36.2% Grade 2. Sparsely granulated corticotroph (Chi square $p = 0.013$) and mature plurihormonal PIT1, sparsely granulated somatotroph and sparsely granulated lactotroph (Chi square $p = 0.005$) were associated with Grade 2. Progression-free

survival was worst for TPIT lineage (Log-rank $p = 0.039$), invasive (Log-rank $p = 0.019$), and Grade 2b (Log-rank $p = 0.012$) tumors.

Conclusion:

In this study, SF1 lineage was the predominant type with significant association between lineage and patient age and clinical presentation. There was a significant association between sparsely granulated subtypes and higher grades. Invasiveness, higher-grade and TPIT lineage were associated with worse outcome.



INTRODUCTION

Pituitary adenomas represent a spectrum of neoplasms originating in the anterior pituitary gland. They represent a significant proportion of intracranial neoplasms (1,2). The patients can be asymptomatic or can present with hormone over secretion associated symptoms or pressure symptoms (3,4). Though initially considered benign, these tumors showcase diverse clinical behaviors, aggressiveness and high rate of recurrence (5).

The previous 2017 WHO classification introduced pituitary adenoma classification based on immunohistochemistry (IHC) for transcription factors and hormonal markers and recognized some subtypes as “high-risk pituitary adenomas(6)”. It has served as a foundation for numerous studies exploring the clinical and pathological features of pituitary adenomas, underscoring the value of immunohistochemical profiling for predicting tumor outcomes (7,8).

Acknowledging the aggressive potential, the recent 2022 World Health Organization (WHO) classification system introduced the nomenclature “Pituitary Neuroendocrine Tumors (PitNET)”, replacing the term “adenoma”. It put forward significant revisions with an emphasis on detailed histological subtyping of PitNETs based on the tumor cell lineage, cell type, and related characteristics according to combined immunohistochemical expression of pituitary hormones (GH, PRL, beta-TSH, beta-FSH, beta-LH, ACTH and alpha-subunit), transcription factors (PIT1, TPIT, SF1, GATA2/3 and ER-alpha) and keratin (CAM 5.2) (9–11). Thus, the PitNETs are currently categorized into four distinct groups: PIT-1-lineage PitNETs, TPIT-lineage PitNETs, SF-1-lineage PitNET, and PitNETs with no distinct lineage

differentiation (9,11). While precise tumor classification is crucial, the assessment of proliferation using mitotic count and Ki-67 index, and radiological or histological evidence of tumor invasiveness have prognostic significance while identifying possible aggressive pituitary adenomas (7,8).

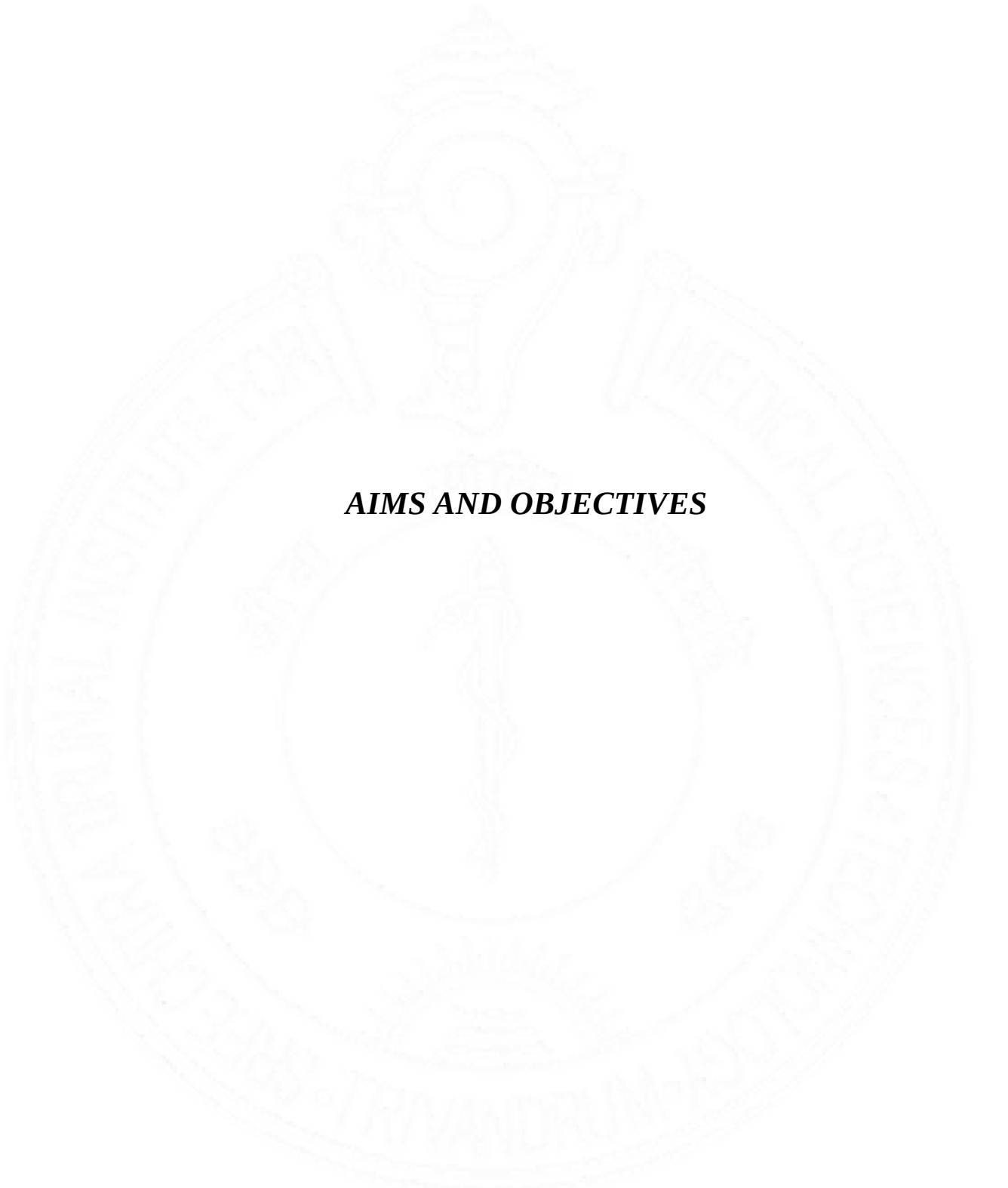
The full clinical and patient outcome implications of the revised 2022 WHO classification for PitNETs is yet to be fully determined. Emerging research has begun to explore the clinical significance of this updated WHO classification system, demonstrating its potential to improve prognostic accuracy and inform treatment strategies. These studies have shown promising correlations between specific PitNET subtypes defined by the 2022 WHO classification and clinical aggressiveness, recurrence rates, and treatment response (12,13).

While existing studies on PitNETs utilizing the 2022 WHO classification have shown promise, most have been conducted in relatively small patient groups and require validation in larger and more diverse populations. Moreover, the most effective treatment approaches for each PitNET subtype defined by the 2022 WHO classification remain an area of active investigation.

Our study aims to contribute to the ongoing research by conducting a comprehensive analysis of PitNETs in a South Indian population, utilizing the 2022 WHO classification. Specifically, this study seeks to provide valuable insights into the practical application of the revised 2022 WHO criteria by investigating the correlations between each PitNET subtype and clinical, pathological, and radiological features, as well as patient outcomes. The findings of this study have implications for future research directions, both basic and translational with

emphasis on the development of personalized treatment strategies based on tumor characteristics.





AIMS AND OBJECTIVES

Aim:

To classify PitNETs based on the 2022 WHO classification and to correlate histopathological subtypes with clinico-radiological features.

Objectives:

1. To classify PitNETs by assessing the expression of pituitary hormones and transcription factors characteristic of a specific lineage via immunohistochemistry.
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REVIEW OF LITERATURE

Pituitary adenomas (PA) are one of the most prevalent intracranial neoplasms. Though considered as rare in the past, advances in imaging and diagnostic techniques have revealed a higher prevalence (1,2). These tumors exhibit a diverse spectrum of clinical presentations, from symptomatic to asymptomatic, posing challenges in accurately determining their true incidence and prevalence. Epidemiological studies, relying on various data sources including autopsy findings, clinical series, and population-based surveys, are crucial for understanding the burden of these tumors on public health.

Epidemiology and Demography:

Global scenario:

A 2004 meta-analysis study by Ezzat et al. revealed an incidence of 16.7% in the general population (14). Several studies, including those by Park et al., Lee et al., and Daly et al., have demonstrated low prevalence rates for pituitary adenomas, with figures as low as 0.09% in Liege, Belgium, and 0.04% in Korea (15,16).

Recent studies show the peak incidence of PA between 40 and 60 years of age and exhibit varying incidence and gender distributions across age groups, different types and races (2,17,18).

Pituitary adenoma in INDIA:

A North India study reported a prevalence of 7.6% (19). Furthermore, research by Dutta P et al. revealed a mean age of diagnosis at 37.1 ± 12.3 years (20).

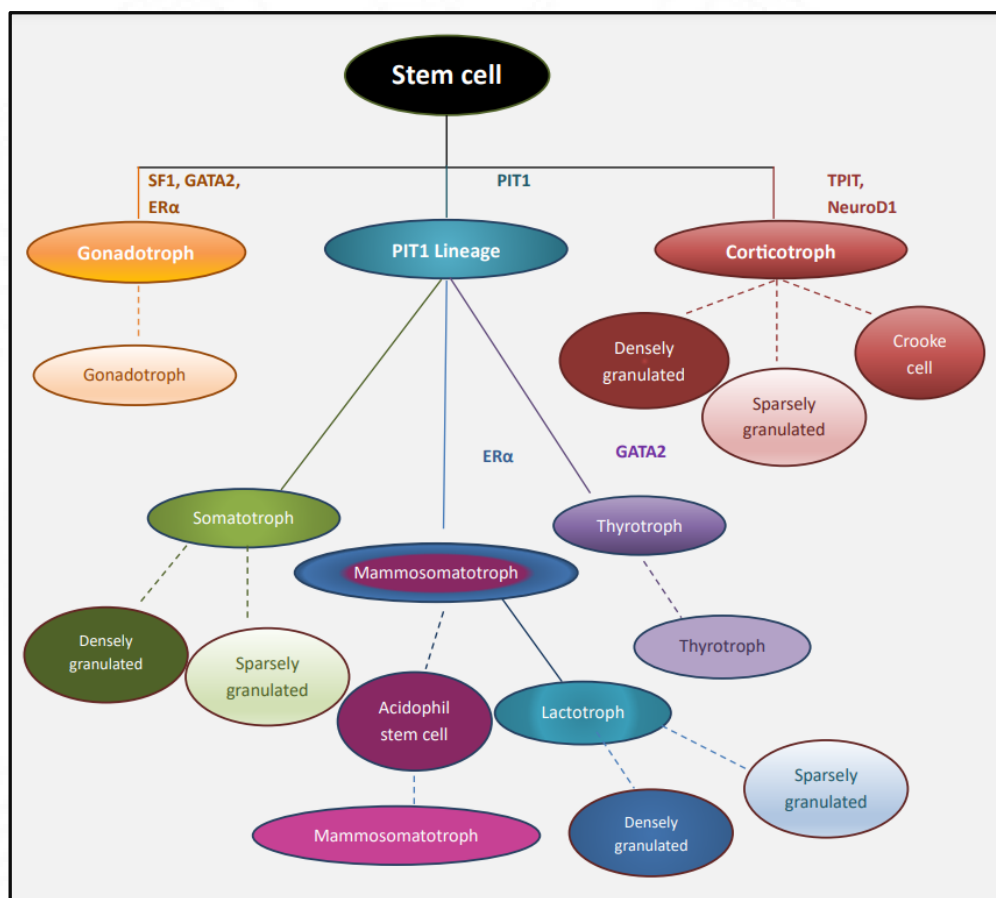
Cellular basis of Pituitary Adenomas:

Pituitary adenomas arise from the clonal proliferation of one or more of specialized hormone-producing cells within the anterior pituitary gland, which originates from oral ectoderm, each expressing distinct hormones regulated by specific transcription factors. For instance, lactotrophs produce prolactin (PRL), growth hormone (GH) is produced by somatotrophs, and proopiomelanocortin (POMC) which is the precursor of adrenocorticotrophic hormone (ACTH), is produced by corticotrophs. Gonadotrophs produce both luteinizing hormone (LH) and follicle-stimulating hormone (FSH), while thyroid-stimulating hormone (TSH) is produced by thyrotrophs (21–23).

The differentiation of pituitary cells is orchestrated by specific transcription factors, which are vital for regulating the genetic expression of hormones. Somatotroph, lactotroph, and thyrotroph development are dependent on Pit-1 (Pituitary-specific positive transcription factor). Corticotroph specialization is controlled by TPit (T-box family member TBX19) and Steroidogenic factor-1 (SF-1) is responsible for the development of gonadotroph lineage (11,13). Corticotroph differentiation requires the coordinated action of Tpit and NeuroD1/beta-2, giving rise to tumors with varying morphologies, including densely granulated, sparsely granulated, and Crooke cell types. Gonadotroph development relies on a combination of transcription factors, such as SF-1 mainly and others like ER α , and GATA-2/3, typically resulting in tumors with relatively consistent morphological features. The Pit-1 lineage is more complex, encompassing the somatotroph, mammosomatotroph, lactotroph, and thyrotroph subtypes, each capable of giving

rise to different types of tumors. Poorly differentiated Pit-1 lineage tumors are hypothesized to originate from a Pit-1 stem cell. It is believed that Acidophil stem cell tumors represent early-stage mammosomatotroph tumors, while null cell tumors may originate from a more undifferentiated stem cell within the adenohypophysis (24).

Figure 1: Cellular basis of Pituitary tumorigenesis



Etiopathogenesis:

Most of the pituitary adenomas arise sporadically with no definite environmental risk factor.

Genetic factors:

Sporadic mutations associated with pituitary tumorigenesis include those affecting signaling pathway genes namely GNAS1 in somatotroph tumors, USP8 in corticotroph tumors and USP48 genes etc. Pituitary adenomas are infrequently linked to germline mutations. These mutations can manifest as part of a syndrome, such as the MEN1 mutation in multiple endocrine neoplasia type 1 (MEN1), or occur in isolation, like the AIP mutation in familial isolated pituitary adenoma (FIPA)(25). Moreover, recent studies have uncovered distinct epigenomic signatures associated with tumor development (25,26).

Clinical Presentation:

The clinical presentation of pituitary adenomas is influenced by both tumor size and their functional status (21,22,27). Based on imaging findings PA are classified into microadenoma (< 10mm), macroadenoma ($\geq$ 10mm) and giant adenoma ($\geq$ 40mm) (28–30). Microadenomas are usually asymptomatic and often detected incidentally on MRI, whereas macroadenomas can cause symptoms due to mass effects and may be associated with hormonal dysfunction (31).

Pituitary adenomas (PAs) are broadly classified as clinically functioning or non-functioning (NF). Both, commonly present with pressure symptoms including visual impairment and headache. Functioning adenomas are characterized by hormone hypersecretion, which can lead to various endocrine disorders. Acromegaly results from excessive growth hormone and insulin-like growth factor 1 (IGF-1) levels. Elevated prolactin levels can cause amenorrhea, galactorrhea, or hypogonadism. Cushing's disease develops due to overproduction of cortisol. In

contrast, non-functioning adenomas typically remain clinically silent in terms of hormonal imbalances, except for potential hyperprolactinemia caused by hypothalamic disruption (32–34).

Pituitary apoplexy, a rare clinical syndrome characterized by sudden bleeding within a pituitary adenoma, was first documented in 1898 (35). It occurs in a small proportion of pituitary adenomas, ranging from 0.6 to 9%, and is often associated with acute hemorrhagic or ischemic events. Common presenting features include headache, nausea, visual disturbances, ophthalmoplegia or paresis, and altered mental state (31,36–39).

Diagnosis of Pituitary adenomas:

Imaging:

Magnetic resonance imaging (MRI) is the cornerstone of pituitary adenoma diagnosis, providing crucial information on the tumor's location, size, invasiveness, and internal characteristics, including cystic, necrotic, or hemorrhagic changes (40,41). The Knosp classification system, based on MRI findings, helps predict the probability of cavernous sinus invasion by pituitary neuroendocrine tumors (PitNETs) (42). Cavernous sinus invasion is a significant obstacle during tumor removal, often resulting in incomplete resection. This increases the risk of tumor recurrence and necessitates further treatment (43).

Endocrine evaluation:

Clinical practice guidelines from The Endocrine Society recommend a thorough hormonal evaluation for all patients with suspected pituitary adenomas, irrespective of presenting symptoms. This evaluation includes measurement of thyroid-stimulating hormone (TSH), free thyroxine (T4), prolactin, follicle-stimulating hormone (FSH), growth hormone (GH), IGF-1, adrenocorticotrophic hormone (ACTH), estradiol, testosterone, and early morning fasting cortisol levels.(21,22,27,31,44,45).

Historical evolution of pathological evaluation and classification of PA**Early Pituitary Tumor Research:**

Following the pioneering work of Minkowski, who linked acromegaly to pituitary tumors, it became evident that neoplasms originating from the anterior pituitary gland are a major cause of significant disease (46). Notably, Harvey Cushing applied the term 'adenoma' even to patients who succumbed to their condition (47). Subsequent research has demonstrated the multifaceted and diverse spectrum of these neoplasms with proliferative and invasive characteristics. These findings have necessitated the development of more refined terminologies and classification systems (48).

WHO Classifications:

The World Health Organization (WHO) classifications of pituitary tumors have been updated several times, culminating in the current 5th edition (2022). A

summary of these changes is presented in table 1(49,50):

Table 1: Evolution of the WHO Classification of Pituitary Adenomas (2004-2022) (Adapted from Lenders et al., 2023)(51).

WHO Classification 2004	WHO Classification 2017	WHO Classification 2022
- Based on morphology and hormonal IHC markers - Three accepted types: Typical pituitary adenoma: Atypical pituitary adenoma: - invasive growth - elevated mitotic index - Ki-67 labelling index >3% - extensive nuclear reactivity for p53 Pituitary carcinoma: - cerebrospinal or distant metastases	- A major paradigm shift; lineage-based classification (transcription factor and hormonal IHC) - The terms 'typical' and 'atypical' omitted - The importance of proliferative markers Ki67 and mitotic count still acknowledged - Recognizes some subtypes of pituitary adenomas as "high-risk pituitary adenomas" due to the clinical aggressive behaviour" - Newly defined: PIT-1 plurihormonal tumours (previously known as 'silent subtype 3') - Pituitary carcinoma: - cerebrospinal or distant metastases	- Nomenclature changes from 'pituitary adenoma' to 'pituitary neuroendocrine tumour' (PitNET) - Transcription factor/ lineage-based diagnostic criteria (SF1, TPIT, PIT1 lineage tumor types with subtypes) 'Pit-1-positive plurihormonal' tumors from 2017 replaced by newly defined two separate tumour types: 'Immature PIT-1 lineage' and 'mature plurihormonal PIT-1 lineage' tumours - Newly defined: tumors with no distinct cell lineage- newly defined type 'plurihormonal tumors' along with previously defined 'null cell tumors' - The term pituitary carcinoma omitted, replaced with 'Metastatic PitNET'

The 2004 WHO classification of pituitary tumors relied primarily on morphology and immunohistochemical (IHC) markers for hormone expression. While there was a significant advance at that time, it had several limitations (49):

- It lacked a robust system for classifying tumors based on cell lineage, hindering accurate prognostication (49).
- Reliance on hormonal immunohistochemistry for lineage determination was less accurate than more recent methods like transcription factor analysis (52).
- The use of proliferative markers, particularly Ki67, for assessing tumor aggressiveness had significant shortcomings including lack of standardized methodology, inter-observer variability and arbitrary cut-off values for defining aggressive and metastatic disease (53).
- P53 immunostaining interpretation also lacked clear guidelines, leading to inconsistent interpretation across laboratories (49,54).

The 2017 WHO classification marked a significant shift in the pathological evaluation of pituitary tumors. A key feature of this classification was the focus on tumor lineage, assessed through immunohistochemical analysis of anterior pituitary hormones and transcription factors. (50). This lineage-based approach allowed for the identification of specific histological subtypes suspected of exhibiting recurrent and/or aggressive clinical behavior. These high-risk subtypes included sparsely granulated somatotroph adenomas, lactotroph adenomas in men, Crooke's cell adenomas, silent corticotroph adenomas, and the newly recognized plurihormonal Pit-1-positive adenoma (55).

A subset of pituitary adenomas show aggressive clinical behaviour, rapid growth, and early recurrence despite adequate treatment (10). Recognizing this aggressive potential, the International Pituitary Pathology Club introduced the entity “Pituitary Neuroendocrine Tumors (PitNET)”, replacing the term “adenoma” (9,11). Following a consensus meeting at the International Agency for Research on Cancer (IARC) in 2017, a “common classification framework” was developed to unify and standardize the reporting of Neuroendocrine Neoplasms (NENs) occurring in various organ systems. NENs represent a spectrum of tumors characterized by cytoplasmic neurosecretory granules and distinctive histological and immunohistochemical features. The term neuroendocrine tumor (NET) designates a subset of these neoplasms that exhibit the potential for metastasis or local invasion, based on the tumor site, type, and tumor grade (11).

Based on these developments, the recent 2021 WHO Classification of CNS tumors 5th edition and the WHO Classification of Endocrine and Neuroendocrine Tumors (2022) introduced the term “Pituitary Neuroendocrine Tumors (PitNET)”, replacing the term “adenoma” (11). It put forward significant revisions with an emphasis on detailed subtyping of PitNETs according to the tumor cell lineage, cell type, and associated features according to pituitary hormones and transcription factors expression in IHC(10). Immunohistochemical assessment for pituitary transcription factors and hormonal markers for accurate diagnosis is mandated in this classification (11,53). The 2022 WHO classification is summarized in table 2:

Table 2: WHO 2022 Classification of PitNETs (modified from Asa et al. 2022)

(11).

Lineage	Type	Subtype	Transcription Factor(s)	Hormone(s)	Keratin (CAM 5.2)
SF1 lineage	Gonadotroph	None	SF1, ER α GATA2/3	α -subunit, β FSH, β LH	Variable
TPIT lineage	Corticotroph	Densely granulated corticotroph	TPIT (TBX19), NeuroD1/ β 2	ACTH and other POMC derivatives	Strong
		Sparsely granulated corticotroph			Variable
		Crooke cell tumour			Intense ring-like perinuclear
PIT1 lineage	Somatotroph	Densely granulated somatotroph	PIT1	GH, α -subunit	Perinuclear
		Sparsely granulated somatotroph		GH	Fibrous bodies (>70%)
	Mammotroph	None	PIT1, ER α	GH (often predominant) PRL, α -subunit	Perinuclear
	Lactotroph	Densely granulated lactotroph	PIT1, ER α	PRL (diffuse cytoplasmic)	Weak or negative
		Sparsely granulated lactotroph		PRL (paranuclear)	Weak or negative
	Thyrotroph	None	PIT1, GATA2/3	α -subunit, β TSH	Weak or negative
	Mature plurihormonal PIT1-lineage	None	PIT1, ER α , GATA2/3	GH (often predominant), PRL, α -subunit, β TSH	Perinuclear
	Immature PIT1-lineage	None	PIT1, ER α , GATA2/3	GH, PRL, α -subunit, β TSH	Focal/Variable
	Acidophil stem cell	None	PIT1, ER α	PRL (predominant), GH (focal/variable)	Scattered fibrous bodies
PitNETs without distinct lineage differentiation	Null cell PitNET/adenoma	None	None	None	Variable
	Plurihormonal	None	Multiple combinations	Multiple combinations	Variable
Multiple PitNETs	Multiple synchronous PitNET/adenomas of distinct lineages	None	PitNET with components of at least two different cell lineage (PIT1, SF1 and TPIT) or a single lineage tumour combined with a null cell tumour		
Metastatic PitNETs	Metastatic PitNETs	None	diagnostic features of PitNET with documented metastasis or discontinuous CNS spread		

Grading of PA:

Trouillas et al. (2013) proposed a French tiered prognostic classification system for PA which suggested grading of PA according to evidence of invasion and proliferation (54). Criteria:

- Invasion- is determined by either or both:
 - MRI - Tumors classified as Grade III or IV according to the Knosp's classification (56).
 - Histology - Invasion of the bone and the respiratory mucosae, extending into the sphenoid sinus(57,58).
- Proliferation- if atleast 2 out of 3 proliferative markers are present:
 - Mitotic count >2 per 10 High power fieldS (HPF)
 - Ki-67 labelling index $\geq 3\%$
 - p53 > 10 strongly positive nuclei per 10 HPF

The prognostic significance of this classification system has been supported by statistical analyses in several studies, including those by Trouillas et al. (2013), Raverot et al. (2017), Lelotte et al. (2018) and Asioli et al. (2019) (7,54,59,60).

Table 3: French five-tiered classification system

Grade	Invasion	Proliferation
Grade 1a	Non-invasive	Non-proliferative
Grade 1b		Proliferative
Grade 2a	Invasive	Non-proliferative
Grade 2b		Proliferative
Grade 3	Metastatic tumor (systemic or cerebrospinal metastasis)	

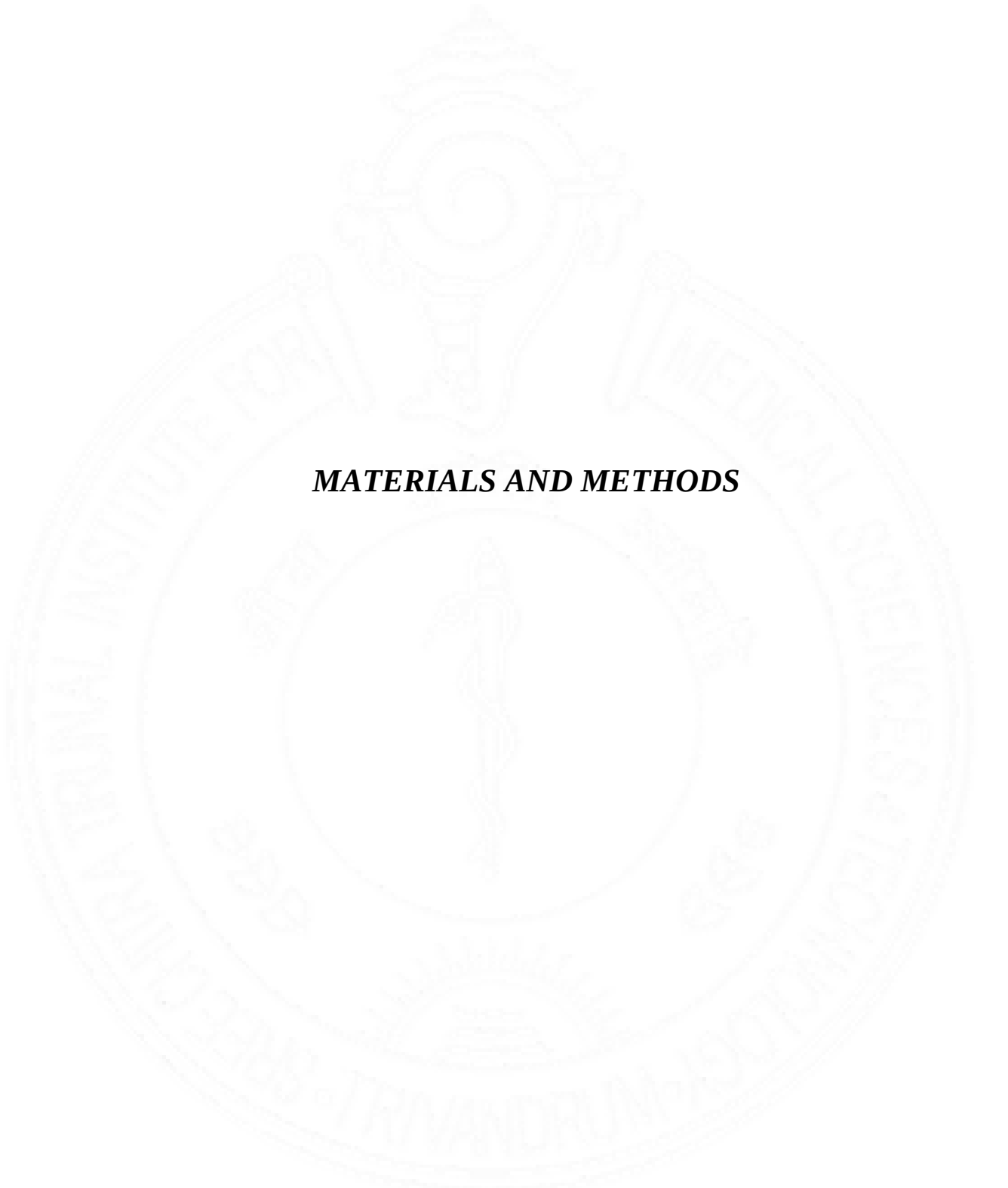
Treatment of PitNETs:

The management of PitNETs is individualized, considering both tumor characteristics and patient symptoms. Treatment strategies range from active surveillance to more interventional approaches like surgery, radiotherapy, and medical therapy (61). Surgery, specifically transsphenoidal resection (endoscopic or microscopic), is generally the first-line treatment (62). Radiotherapy plays a role in preventing recurrence post-surgically but is primarily used for patients who are not fit for surgery (63). For recurrent PitNETs, and considering the risks associated with radiotherapy, medical therapies offer a valuable alternative. Dopamine agonists, somatostatin analogs, and temozolomide (TMZ) have shown the most promise in this context (53,64,65).

Aggressive PitNETs:

A subset of pituitary adenomas shows aggressive clinical behaviour, rapid growth, and early recurrence despite adequate treatment(66). Research by Thapar et al. and Scheithauer et al. indicates a correlation between elevated Ki67 proliferation index and P53 positivity with aggressive behavior and tumor invasion(66,67). The 2017 WHO classification's lineage-based diagnostic system enabled the recognition of specific histological subtypes considered 'high-risk,' including sparsely granulated somatotroph adenomas, lactotroph adenomas in males, Crooke's cell adenomas, silent corticotroph adenomas, and the recently defined plurihormonal Pit-1-positive adenomas (50,55). While some studies suggest a prognostic role for these subtypes and morphological features, the evidence remains inconsistent and inconclusive.

For aggressive PitNETs, characterized by rapid growth, local invasion, resistance to standard therapies, or multiple recurrences despite optimal treatment, TMZ is the current first-line treatment. Emerging therapeutic options include peptide receptor radionuclide therapy, anti-angiogenic therapies targeting tyrosine kinase inhibitors, vascular endothelial growth factor receptors (VEGF-R), mammalian target of rapamycin (mTOR) inhibitors, and, more recently, immune checkpoint inhibitors (68–71).



MATERIALS AND METHODS

STUDY DESIGN

Retrospective study

STUDY PERIOD

1st January 2016 to 31st December 2023 (8 years)

INCLUSION CRITERIA

All pituitary adenoma/ PitNETs diagnosed from 01 January 2016 to 31 December 2023, with adequate tissue in paraffin blocks for IHC.

EXCLUSION CRITERIA

1. Cases with tissue blocks inadequate for IHC
2. Extensive necrosis/ apoplexy with scant viable tumor tissue to perform IHC

STUDY SETTING:

All cases of Pituitary adenoma/ PitNET, fulfilling the inclusion criteria received and diagnosed in the study period at the Department of Pathology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST). Sample size is 514 cases.

ETHICAL COMMITTEE CLEARANCE:

Prior to commencement, the study protocol was submitted to and approved by the SCTIMST Institutional Ethics Committee (IEC Approval No: SCT/IEC/2205/MARCH/2024).

DATA COLLECTION:

A total of 514 cases that met the predefined inclusion criteria were selected for this study. Histopathology reports of each of these cases were retrieved from the archives in Pathology department (electronic medical records system of Pathology reports, SCTIMST). Existing slides (haematoxylin-eosin stained and
SCTIMST, Trivandrum

immunohistochemical) from surgical resections or biopsy specimens were re-evaluated. Additional immunohistochemical testing was performed in tissue sections of necessary cases, according to standardized protocols, to complete the PitNET IHC panel:

1. Transcription factors specific to lineage: PIT1, SF1, TPIT, GATA2, ER-alpha
2. Pituitary hormonal markers: GH (Growth Hormone), PRL (Prolactin), ACTH, TSH-beta, FSH-beta, LH-beta, LH-alpha
3. Others: Proliferation marker (Ki-67), p53 and cytokeratin- CAM 5.2

The data was collected using a proforma which included:

- a) Histopathology: PitNET types, subtypes, grade (71), invasion, apoplexy
- b) Immunohistochemistry: Transcription factors, hormonal markers, p53, CAM 5.2-positive/negative, Ki67/ MIB labelling index: in percentage
- c) Clinical and radiological features: age, gender, clinical manifestations-functional/non-functional, invasive/non-invasive
- d) Follow up:

Follow-up period (months/years): all available follow-up from the date of first surgery until 31 July 2024 was collected from electronic medical records.

Follow up definitions (59):

Recurrence was defined as: either radiological evidence of tumor on MRI and, or elevated plasma hormone concentrations and histopathological evidence of adenoma in patients who attained remission.

In recurrent cases, the time to recurrence from initial diagnosis (months/ years) was collected from electronic medical records.

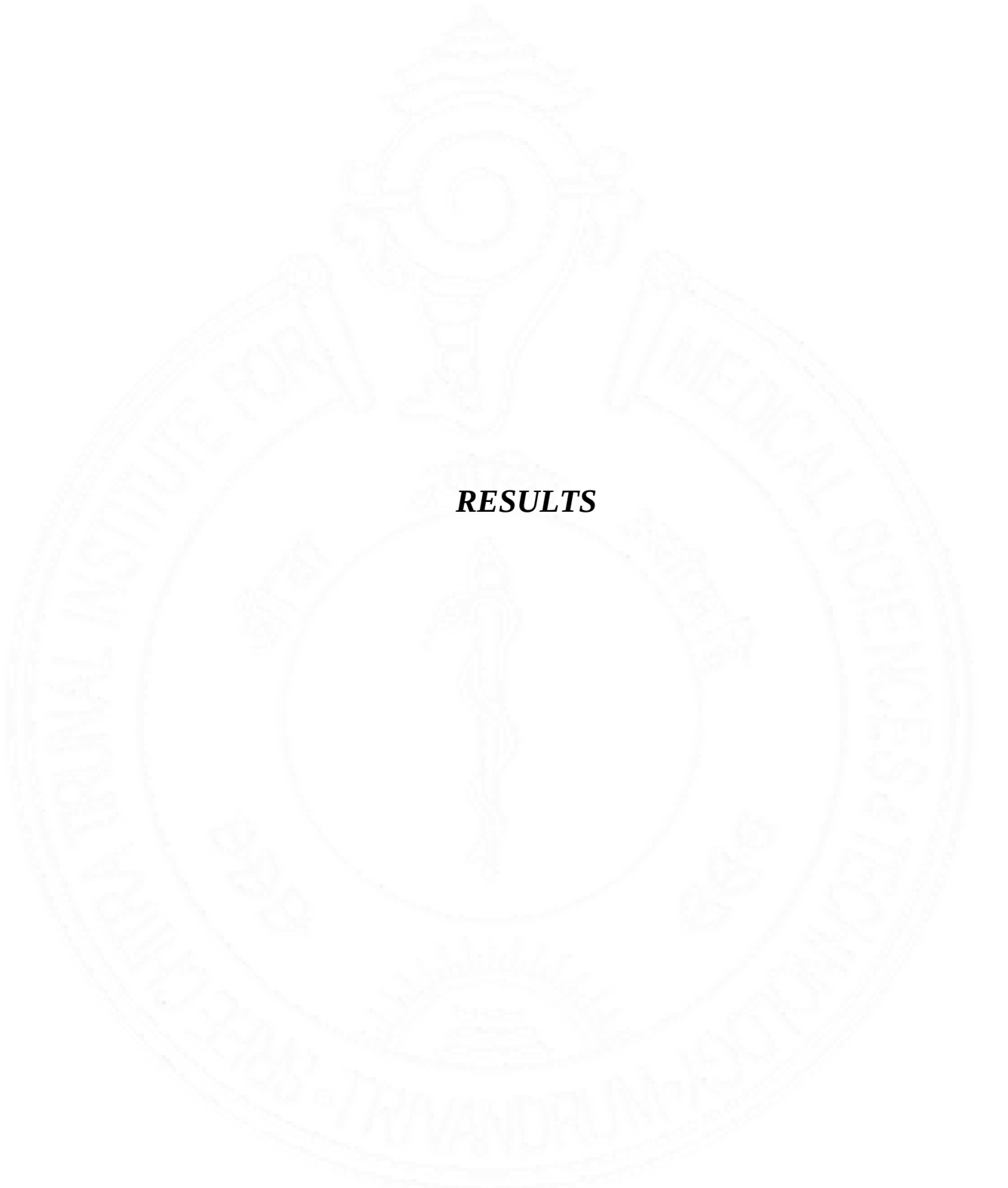
Complete remission was defined as: no evidence of disease during follow-up (no clinical symptoms, normal plasma hormone levels, and no visible radiological residual tumor)

Progression of the tumor was determined by one or more of the following criteria: an increase in the size of the residual tumor as observed on MRI, a rise in plasma hormone levels, the identification of new invasive characteristics, or evidence of increased proliferation through histopathological examination

In such cases, the time to progression from initial diagnosis (months/ years) was collected from electronic medical records.

STATISTICAL ANALYSIS:

Data were de-identified and organized in a custom Excel spreadsheet before being statistically analyzed using a trial version of SPSS software (version 30.0.0, IBM Corp.) Categorical variables are presented as frequencies and percentages, while continuous variables are presented as means with standard deviations. The relationships between various clinicopathological factors, including tumor subtype, grading, invasiveness, clinical presentation, imaging results, and treatment modalities, were assessed using suitable statistical techniques. Differences in categorical variables were evaluated using the Chi-square test. Survival rates were analyzed using Kaplan-Meier curves, and Cox proportional hazards regression was employed for survival analysis. Results were deemed statistically significant if the p-value was below 0.05.



RESULTS

Demographics

The study included 514 patients, evenly distributed between males (n=257) and females (n=257), with ages ranging from 6 to 82 years (mean: 46.04 ± 13.49 years).

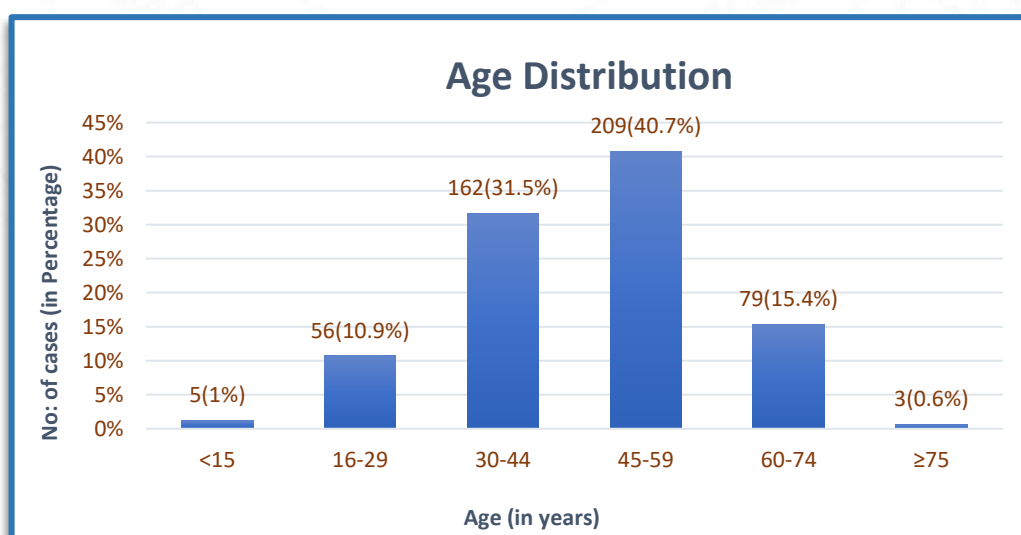
Table 4: Age wise distribution of cases

	Minimum	Maximum	Mean	Std. deviation
Age	6	82	46.04	13.49

Distribution of cases based on age:

The age ranges were categorized as 15 to 29 years, 30 to 44 years, 45 to 59 years, 60 to 74 years, and 75 years or older. The 45-59 year age bracket contained the highest percentage of patients, accounting for 40.7%, while 31.5% of patients were in the 30-44 year age range.

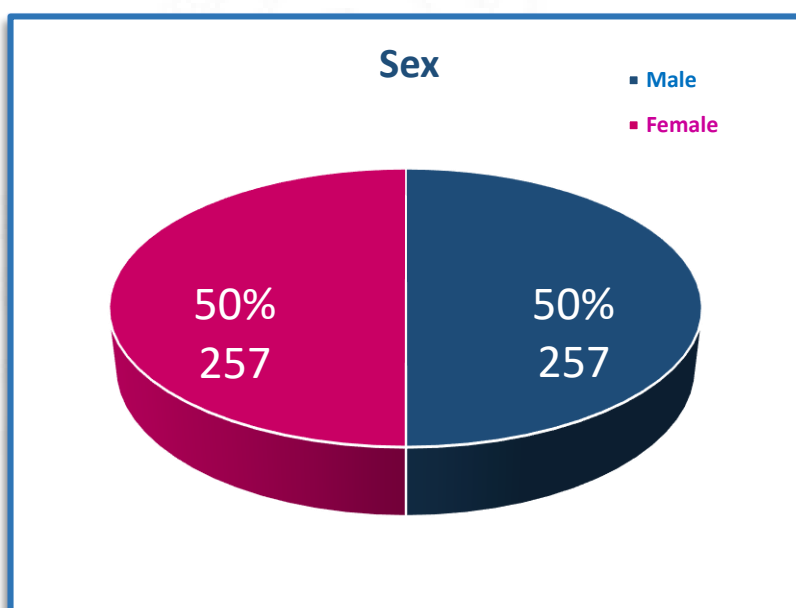
Figure 2: Age group wise distribution of cases



Gender wise distribution of cases:

Of the 514 patients studied, 257 (50%) patients were females, and 257 (50%) patients were males.

Figure 3: Gender wise distribution of cases



Age and Sex Correlation:

Out of the 514 patients studied, nearly equal distribution of cases was noted in both the sex in all age groups, except for more than 75-year-old age group, with only males. Male patients had a mean age of 46.5 ± 13.4 years, while the female patients' mean age was 45.6 ± 13.6 years. Patient age and gender did not demonstrate a statistically significant correlation (Chi-square $p = 0.38$).

Table 5: Age and sex correlation

Age (years)	Sex		Total
	Female	Male	
≤15	3	2	5
16 – 29	32	24	56
30 – 44	77	85	162
45 – 59	108	101	209
60 – 74	37	42	79
≥75	0	3	3
Total	257	257	514
Chi square p = 0.382			

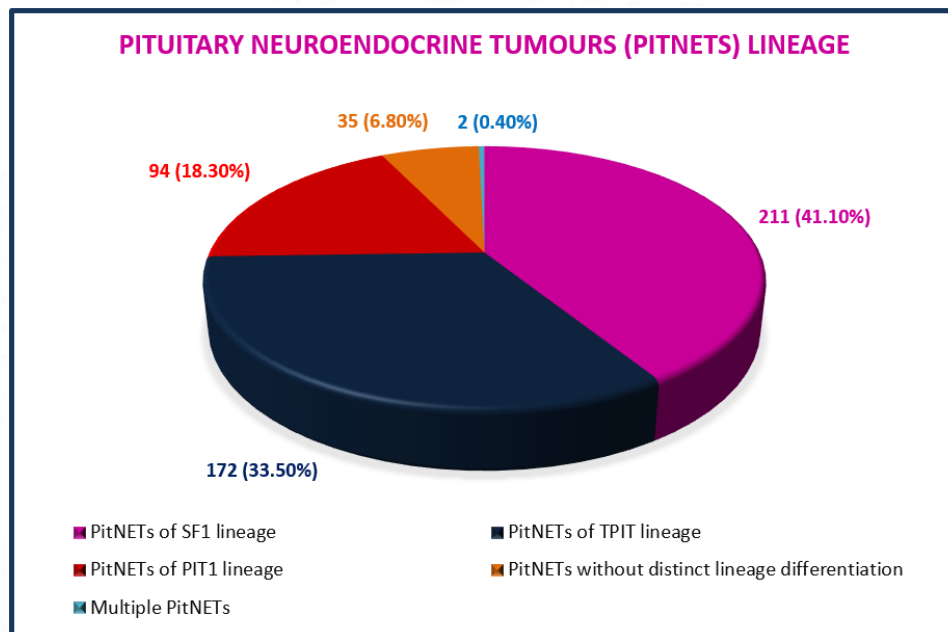
Clinical Presentation:

Most patients (66.5%, n=342) presented with pressure symptoms of visual disturbance and headache. Endocrine symptoms including acromegaly, Cushing disease, galactorrhea, menstrual irregularities, sexual dysfunction and features of hyperthyroidism were observed in 7.4% (n=38) and 23.9% (n=123) presented with both.

Tumor Classification:

Based on the Transcription factor IHC, the PitNETs were grouped into five types, in accordance with WHO 2022 classification:

Figure 4: WHO 2022 classification-based distribution of cases



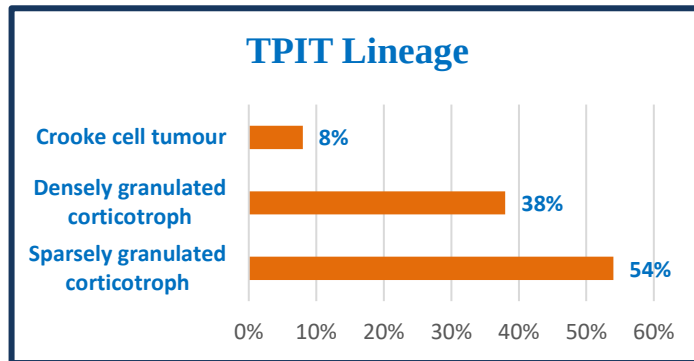
1. *SF1 Lineage PitNETs:*

Represented the largest group (41.1%, n=211).

2. *TPIT Lineage PitNETs:*

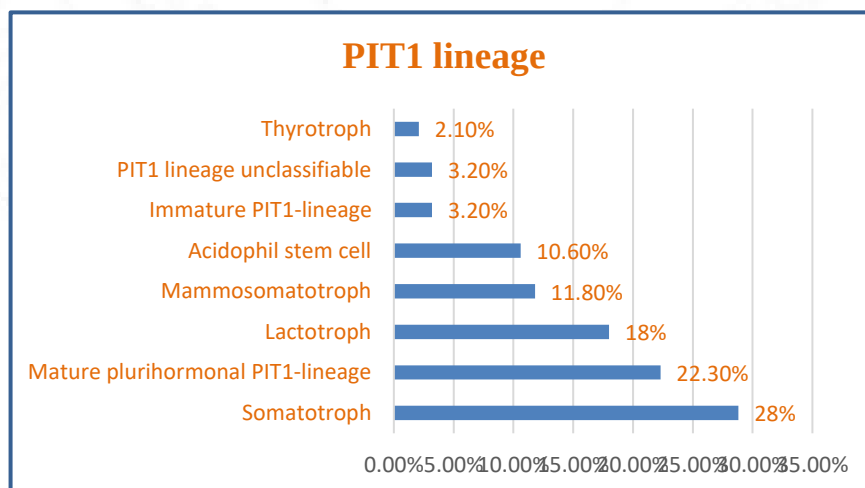
Accounted for 33.5% (n=172). Sparsely granulated was the predominant subtype (94, 54%), followed by densely granulated (66, 38%) and Crooke cell tumor (14, 8%).

Figure 5: Distribution of TPIT Lineage PitNETs



3. PIT1 Lineage PitNETs: Comprised 18.3% (n=94). The majority were Somatotroph (27, 28%), followed by Mature plurihormonal PIT1-lineage (21, 22.3%), Lactotroph (17, 18%), Mammosomatotroph (11, 11.8%), Acidophil stem cell (10, 10.6%), Immature PIT1-lineage (3, 3.2%), PIT1-lineage unclassifiable (3, 3.2%) and Thyrotroph (2, 2.1%) subtypes. Out of the 27 somatotroph PitNETs, 20 (74%) were sparsely granulated and 7 (26%) densely granulated. Among the 17 lactotroph PitNETs, 14 (82.4%) were densely granulated and 3 (17.6%) were sparsely granulated.

Figure 6: Distribution of PIT1 lineage PitNETs



4. PitNETs without Distinct Lineage Differentiation: Included 6.8% (n=35), predominantly Plurihormonal (91.4%,32/35) and three cases of Null cell adenomas (8.6%). 78% (25/32) of plurihormonal tumors co-expressed PIT1/SF1 with 9.4% each (3/32) of TPIT/PIT1 and TPIT/SF1.

5. Multiple PitNETs: Two cases (0.4%) were multiple synchronous adenomas of distinct lineages: one with PIT1 and TPIT and another with SF1 and PIT1 expressions.

Sex distribution among PitNETs:

SF1 and PIT1 lineage PitNETs occurred significantly more commonly in males (71.6% and 58.5% respectively) while TPIT lineage in females (83.2%) (Chi-square $p < 0.001$).

Figure 7: Sex distribution of different types of PitNETs

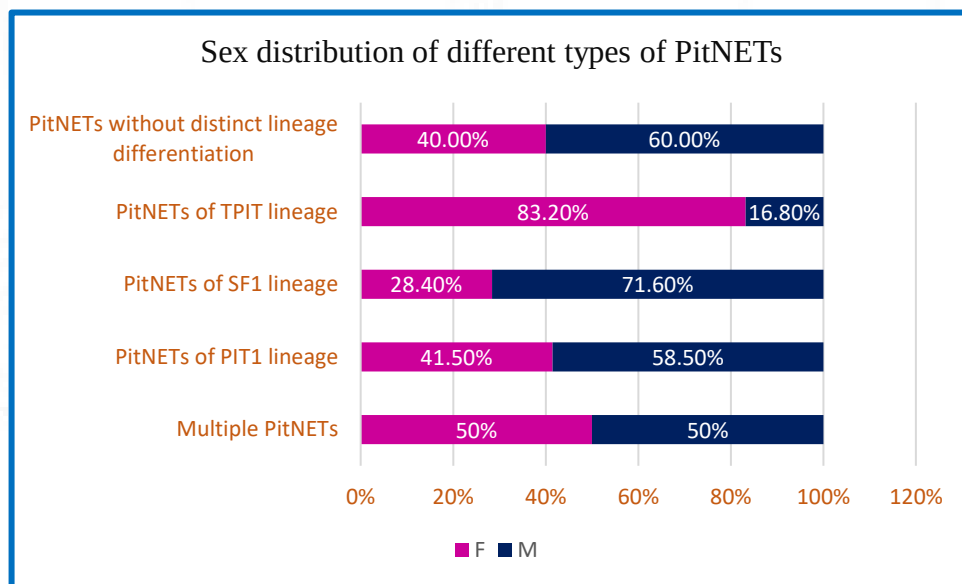


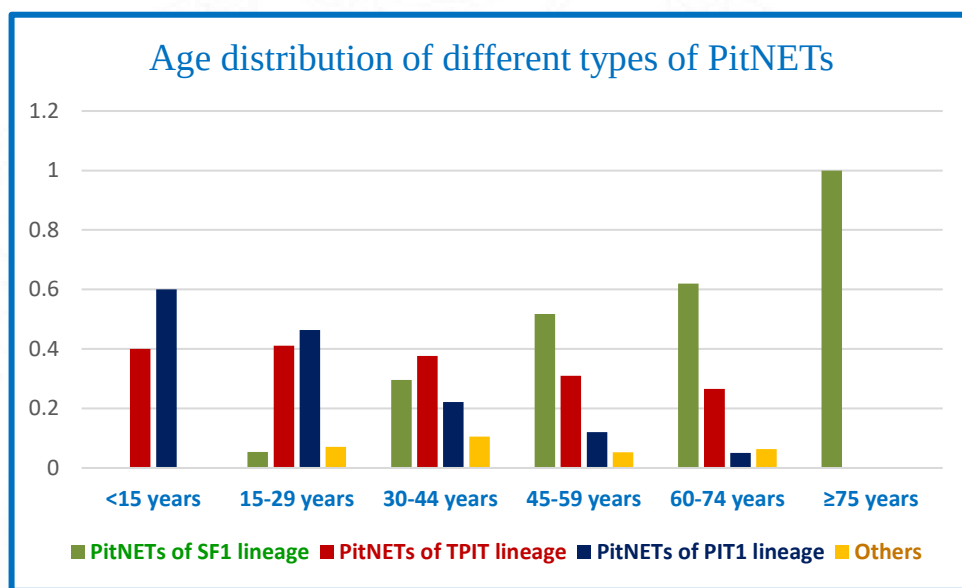
Table 6: WHO 2022 classification and gender-based distribution of cases

Lineage	Type	Subtype	Males	Females	Total number	Percentage
SF1 lineage	Gonadotroph	None	151	60	211	41.1%
TPIT lineage	Corticotroph	Densely granulated corticotroph	14	51	65	12.6%
		Sparsely granulated corticotroph	9	84	93	18.1%
		Crooke cell tumour	6	8	14	2.7%
PIT1 lineage	Somatotroph	Densely granulated somatotroph	4	3	7	1.4%
		Sparsely granulated somatotroph	11	9	20	3.9%
	Mammotroph	None	7	4	11	2.1%
	Lactotroph	Densely granulated lactotroph	10	4	14	2.7%
		Sparsely granulated lactotroph	1	2	3	0.6%
	Thyrotroph	None	0	2	2	0.4%
	Mature plurihormonal PIT1-lineage	None	13	8	21	4.1%
	Immature PIT1-lineage	None	0	3	3	0.6%
	Acidophil stem cell	None	6	4	10	1.9%
	PIT1 lineage unclassifiable	None	3	0	3	0.6%
PitNETs without distinct lineage differentiation	Null cell PitNET/adenoma	None	1	2	3	0.6%
	Plurihormonal	None	20	12	32	6.2%
Multiple PitNETs	Multiple synchronous PitNET/adenomas of distinct lineages	None	1	1	2	0.4%

Age distribution among PitNETs:

The most prevalent PitNETs in different age-groups were: <30 years - PIT1 lineage (44.3%), 30-44 years - TPIT lineage (37.7%) and >45 years - SF1 lineage (55%). There was a significant difference between the PitNET type and patient age (Chi-square $p < 0.001$).

Figure 8: Age distribution of different types of PitNETs



Clinical presentation of different PitNETs:

Out of 514 cases, the majority presented initially with pressure symptoms ($n=397$, 77.2%), followed by 67 (13%) with both endocrine and pressure symptoms. 36 cases (7%) presented with endocrine symptoms alone, while 14 (2.7%) cases incidentally detected were asymptomatic. The most common pressure symptom was visual disturbances followed by headache. Others included: nausea, vomiting and dizziness. Most common endocrine symptom was acromegaly, followed by Cushing disease.

Pressure symptoms were the most common presentation in SF1 (88%), TPIT (61.6%), and other PitNETs, while majority of PIT1 lineage tumors had acromegaly and pressure symptoms (44.7%). These differences across lineages were statistically significant (Chi-square $p < 0.001$).

Tumor Size:

Tumor size ranged from 0.3 to 7.8 cm (mean: 3.19 ± 1.27 cm).

Table 7: Tumor size distribution in study population

Sample	Minimum	Maximum	Mean	Std. deviation
Total sample (n=514)	0.3cm	7.8cm	3.19cm	1.27cm
SF1 Lineage	1.4cm	6.2cm	3.25cm	0.89cm
TPIT Lineage	0.3cm	7.8cm	3.29cm	1.48cm
PIT1 Lineage	0.5cm	7.7cm	2.96cm	1.52cm
Others	0.9cm	6.4cm	2.93cm	1.34cm

Macroadenomas constituted 75% (n=384), giant adenomas 21.5% (n=110), and microadenomas 3.5% (n=18). Giant adenomas were most common in TPIT lineage tumors (40%), followed by SF1 lineage (35%). Among macroadenomas, the majority were SF1 (44.8%) and TPIT lineages (29.7%). 72% of microadenomas belonged to TPIT lineage. (Chi-square $p < 0.001$)

Table 8: Tumor size-based classification of PitNETS

Tumor Size	PitNETs of SF1 lineage	PitNETs of TPIT lineage	PitNETs of PIT1 lineage	PitNETs without distinct lineage differentiation	Multiple PitNETs	Total
Macroadenoma	172	114	71	26	1	384
Giant adenoma	39	44	19	8	0	110
Microadenoma	0	13	4	1	0	18
Size data not available	0	1	0	0	1	2
Total	211	172	94	35	2	514
Chi-square $p < 0.001$						

Tumor Invasion:

Invasion of cavernous or sphenoid sinus was observed in 36.2% (n=186/514); was significantly associated with TPIT (n=80/186, 43%) and SF1 (n=53/186, 28.5%) (Chi-square $p < 0.001$) lineage tumors.

Table 9: Tumor invasiveness across PitNET lineages

Lineage	Non invasive	Invasive	Total
Multiple PitNETs	2	0	2
PitNETs of PIT1 lineage	52	42	94
PitNETs of SF1 lineage	158	53	211
PitNETs of TPIT lineage	92	80	172
PitNETs without distinct lineage differentiation	24	11	35
Total	328	186	514
Chi-square $p < 0.001$			

Apoplexy:

Apoplexy was observed in 8.4% (n=43/514) tumors, most frequently in TPIT (12.8%) and SF1 (8%) lineage tumors. The association between apoplexy and tumor lineage was significant (Chi-square $p = 0.004$).

Table 10: Apoplexy across PitNET Lineages

Lineage	Apoplexy		Total
	Absent	Present	
PitNETs of TPIT lineage	150	22	172
PitNETs of SF1 lineage	194	17	211
PitNETs of PIT1 lineage	91	3	94
Multiple PitNETs	1	1	2
PitNETs without distinct lineage differentiation	35	0	35
Total	471	43	514
Chi-square $p = 0.004$			

Grading:

The most common tumor grade was Grade 1a, observed in 50.8% of cases. Subsequent grades were Grade 2a (30%), 1b (13%), and 2b (6.2%).

Grade distribution varied significantly across lineages ($p < 0.001$). SF1 lineage (58.8%) and TPIT lineage (48.2%) were predominantly Grade 1a, while PIT1 lineage belonged to Grade 1a and 2a (34% each). Among TPIT lineage tumors, Grade 2 was significantly associated with the sparsely granulated subtype (Chi-square $p = 0.013$). In PIT1 lineage, mature plurihormonal, sparsely granulated somatotroph and lactotroph subtypes were associated with higher-grade tumors (Chi-square $p = 0.005$).

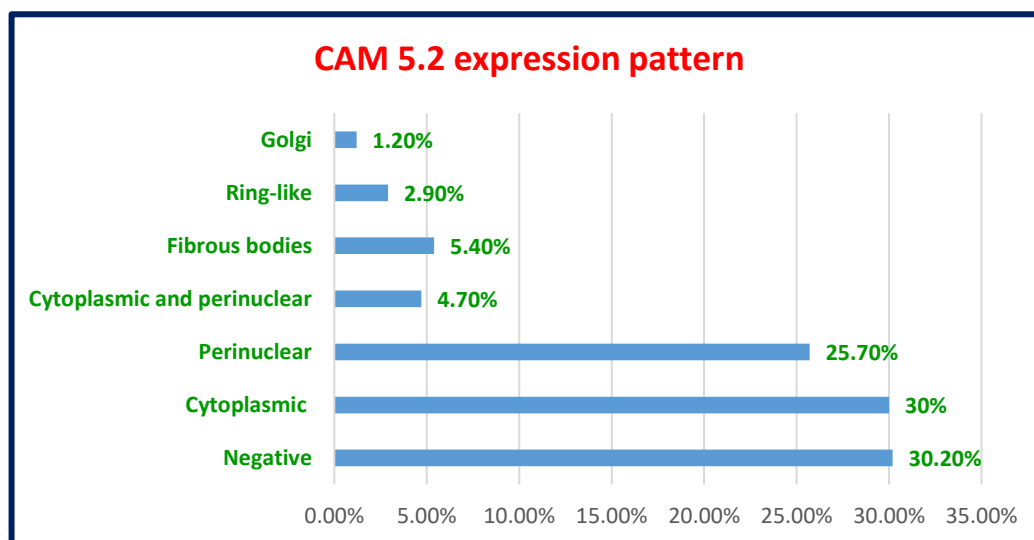
Table 11: Tumor grades across PitNET Lineages

Lineage	Grade 1a n=261	Grade 1b n=67	Grade 2a n=154	Grade 2b n=32	Total n=514
PitNETs of SF1 lineage	124 (58.8%)	34 (16%)	40 (19%)	13 (6.2%)	211
PitNETs of TPIT lineage	83 (48.2%)	9 (5.2%)	73 (42.4%)	7 (4.1%)	172
PitNETs of PIT1 lineage	32 (34%)	20 (21.4%)	32 (34%)	10 (10.6%)	94
PitNETs without distinct lineage differentiation	20 (57.2%)	4 (11.4%)	9 (25.7%)	2 (5.7%)	35
Multiple PitNETs	2 (100%)	0	0	0	2
Chi-square p <0.001					

Immunohistochemical Findings:**CAM5.2 Expression Patterns:**

CAM5.2 was expressed in 69.8%. The patterns were: cytoplasmic (30%), perinuclear (25.7%), fibrous bodies (5.4%), both cytoplasmic and perinuclear (4.7%), ring-like (2.9%), and Golgi (1.2%). In TPIT and PIT1 lineage tumors, there was a significant difference in CAM5.2 patterns between the subtypes (Chi-square p < 0.001).

Figure 9: CAM 5.2 expression pattern in study population



MIB-1 Labelling index:

Across the 514 cases, the MIB-1 Labelling index (LI) varied between 1 to 10 % (mean: 2.3 ± 1.4 %). Majority of cases, 51.8% (n=266/514), had MIB-1 labeling index of 3–5%, 33.9% (n=174/514) of <3% and 14.4% (74/514) of 6-10%, There was significant difference in MIB-1 LI across lineages with majority of cases with >3% MIB1 LI belonging to SF1 lineage (43%, 147/340) followed by TPIT (31%, 104/340) and PIT1 lineages (20%, 68/340) (Chi-square $p < 0.005$).

Table 12: MIB-1 Labelling index across PitNET lineages

Lineage	MIB-1 LI			Total
	<3%	3-5%	6-10%	
SF1 Lineage	64	128	19	211
TPIT Lineage	68	76	28	172
PIT1 Lineage	26	45	23	94
Without lineage differentiation	15	16	4	35
Multiple PitNETs	1	1	0	2
Total	174	266	74	514
Chi-square $p < 0.005$				

p53:

Out of 514 cases, 100 (19.5%) showed positivity for p53 (>10/10hpf),. p53 was negative in 414 cases (80.5%).

ATRX:

Only TPIT lineage tumors were tested for ATRX expression. Out of the 172 cases of TPIT Lineage tumors, interpretable ATRX results were available for 166 cases, of which only one showed loss of nuclear expression which belonged to densely granulated corticotroph PitNET, grade 2b.

Follow-Up and Outcome:

433 cases with initial surgery done within the study period were included for follow-up; mean duration of follow-up was 27.56 ± 26.25 months and median duration was 19 months. 16.9% (n=73) experienced an event during follow-up, including recurrence in 11.8% (n=51) and progression in 5.1% (n=22).

Kaplan-Meier analysis:

Kaplan-Meier analysis revealed that median progression-free survival (PFS) varied significantly across PitNET lineages: TPIT- 64 months, SF1- 82 months, PIT1 and others- not reached (log-rank $p=0.039$). Tumor invasiveness – worse for invasive tumors (log-rank $p=0.019$), and tumor grade – worst for Grade 2b (log-rank $p=0.012$) were also associated with shorter PFS. There were no significant differences in the PFS based on tumor size (log-rank $p=0.172$).

Figure 10: Kaplan-Meier plots representing the probability of progression/ recurrence free survival (PFS) according to different PitNET lineages

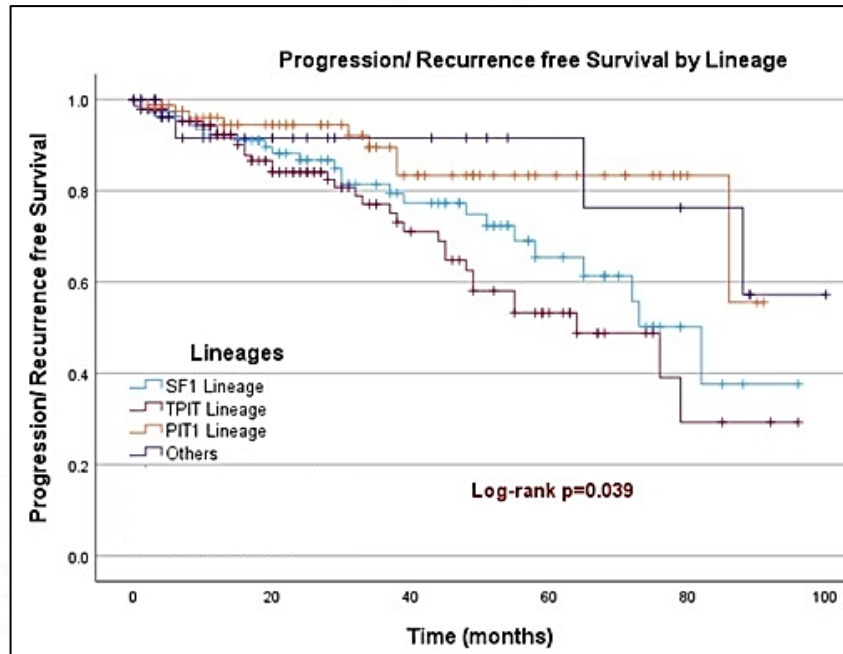


Figure 11: Kaplan-Meier plots representing the probability of progression/ recurrence free survival (PFS) according to invasiveness

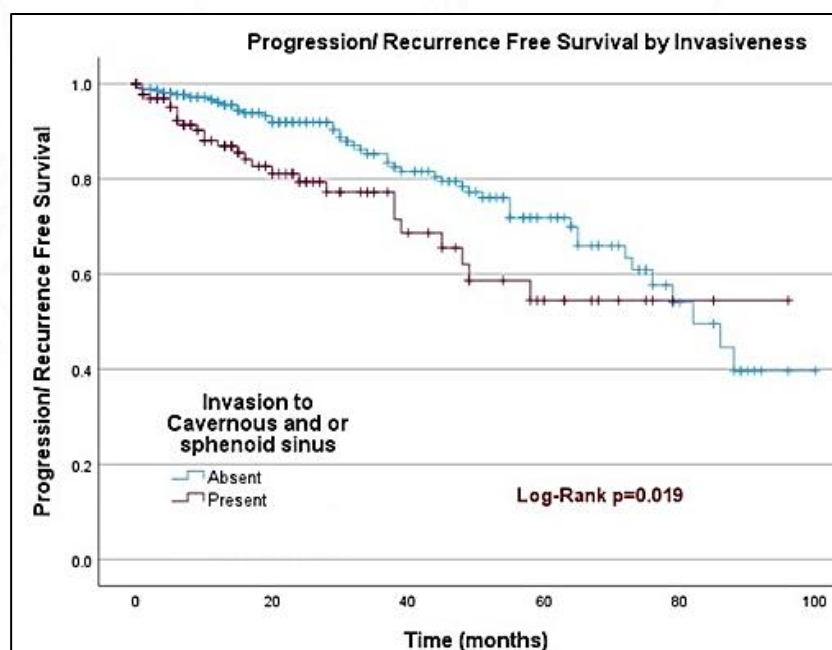


Figure 12: Kaplan-Meier plots representing the probability of progression/ recurrence free survival (PFS) according to tumor grade

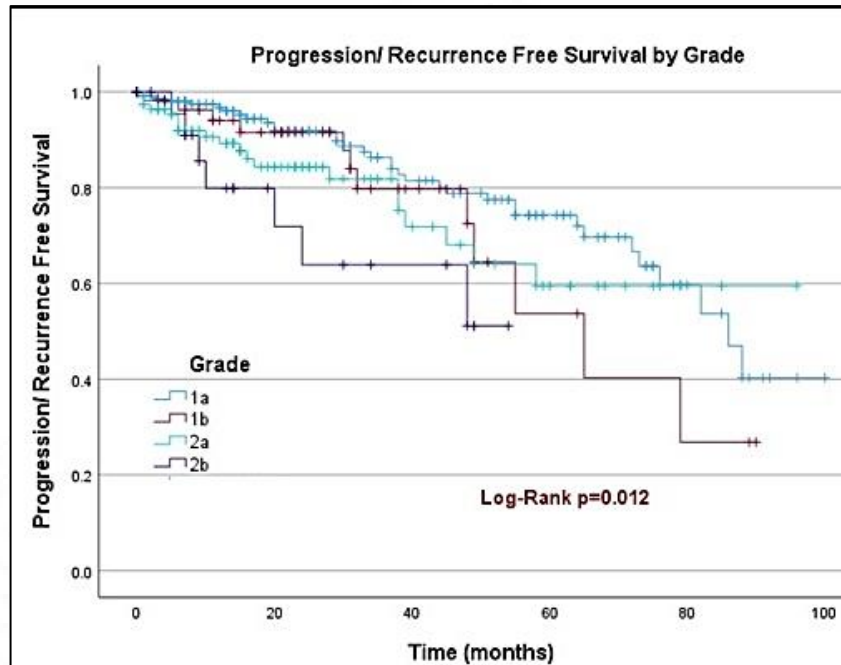
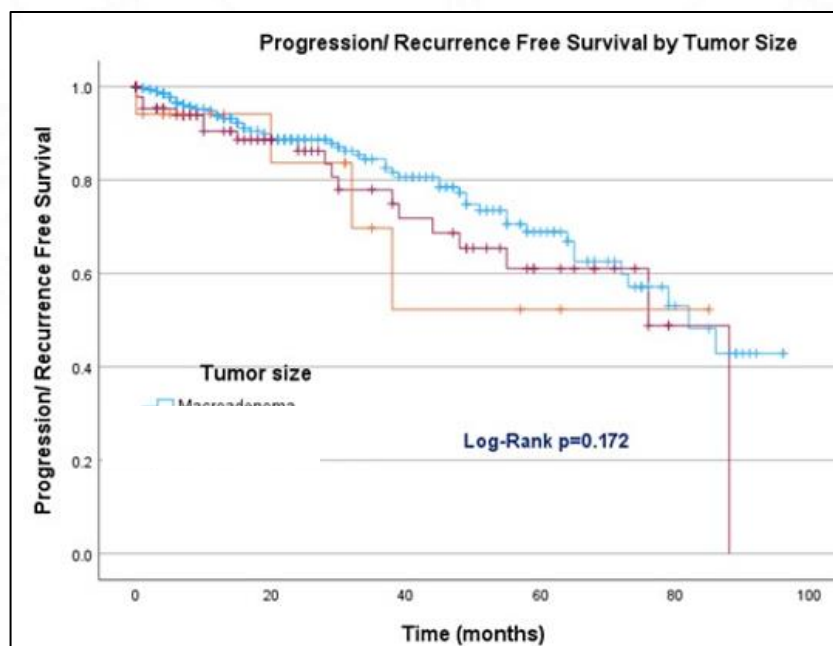


Figure 13: Kaplan-Meier plots representing the probability of progression/ recurrence free survival (PFS) according to tumor size



Cox Univariate and multivariate analysis:

This study aimed to identify independent predictors of progression or recurrence following surgery for PitNETs.

Univariate Cox regression analysis:

This test was performed to assess the association of age, sex, functional status, size of the tumor, invasiveness, Ki-67 index, and tumor grade with progression or recurrence. Significantly higher progression/ recurrence rates were noted across PitNET lineages: TPIT (log-rank $p = 0.005$) and SF1 (log-rank $p = 0.050$), invasive tumors (log-rank $p = 0.021$), and higher-grade tumors: Grade 2b (log-rank $p = 0.014$).

Multivariate Cox regression analysis:

After combining these variables in Cox multivariate analysis, only the PitNET lineage was identified as the sole independent predictor of PitNET progression or recurrence. TPIT (Hazard ratio=4.3) and SF1 (Hazard ratio=4.6) lineages were significantly associated with tumor progression/recurrence (log-rank $p = 0.007$).

Table 13: Univariate Cox regression analyses for progression or recurrence

Variables		Univariate analysis	
		Hazard ratio [95% CI]	Log-rank p value
Gender	Male (Vs. Female)	0.894 (0.563-1.417)	0.633
Age group	30-44 years (Vs. 0-29 years)	0.672 (0.368-1.226)	0.195
	45-64 years (Vs. 0-29 years)	0.443 (0.238-0.823)	0.010
	65-100 years (Vs. 0-29 years)	0.642 (0.146-2.814)	0.556
Hormonal status	Functioning (Vs. Non-functioning)	1.015 (0.593-1.739)	0.956
Tumor size	Microadenoma (Vs. Macroadenoma)	1.549 (0.558-4.300)	0.400
	Giant adenoma (Vs. Macroadenoma)	1.382 (0.814-2.347)	0.231
Invasiveness	Invasion (Vs. Non-invasive)	1.762 (1.090-2.849)	0.021
Proliferation	MIB 1 LI $\geq 3\%$ (Vs. $<3\%$)	1.569 (0.919-2.677)	0.099
Tumor Grade	Grade 1b (Vs. grade 1a)	1.462 (0.754-2.833)	0.261
	Grade 2a (Vs. grade 1a)	1.565 (0.897-2.731)	0.115
	Grade 2b (Vs. grade 1a)	2.838 (1.238-6.504)	0.014
Tumor lineage	SF1 Lineage (Vs. PIT1)	2.139 (1.001-4.569)	0.050
	TPIT Lineage (Vs. PIT1)	2.873 (1.376-5.998)	0.005
	Other Lineages (Vs. PIT1)	1.065 (0.324-3.501)	0.917

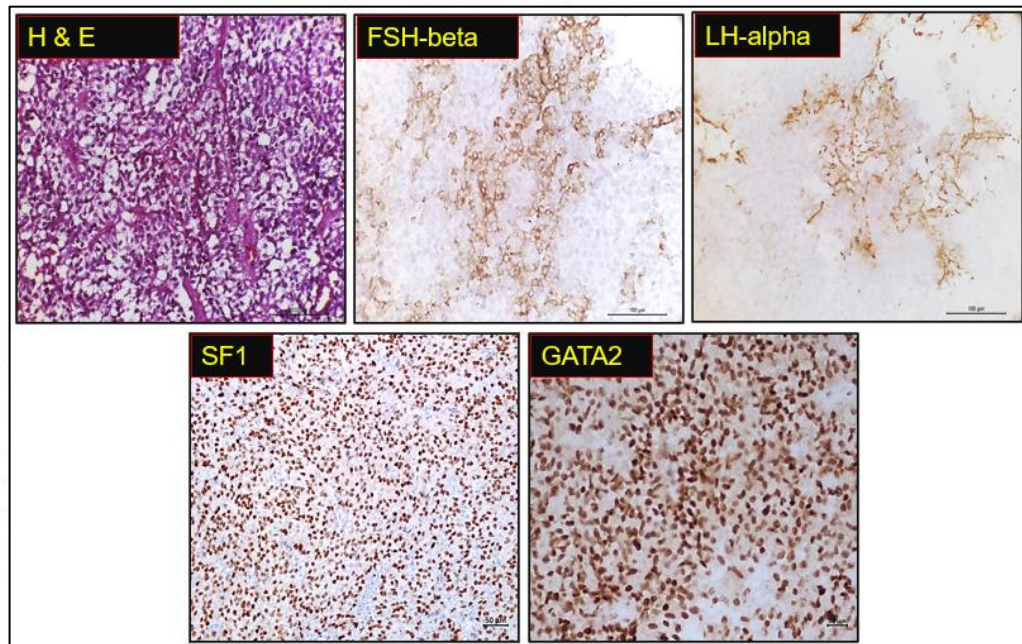
Table 14: Multivariate Cox regression analyses for progression or recurrence

Variable		Hazard ratio	95% CI	Log-rank p value
Lineage	TPIT Vs. PIT1	4.251	1.814-9.963	0.002
	SF1 Vs. PIT1	4.621	1.735-12.306	0.001

Representative cases

SF1 Lineage:

Figure 14: Case 1- Gonadotroph PitNET



TPIT Lineage

Figure 15: Case 2- Densely granulated corticotroph tumor

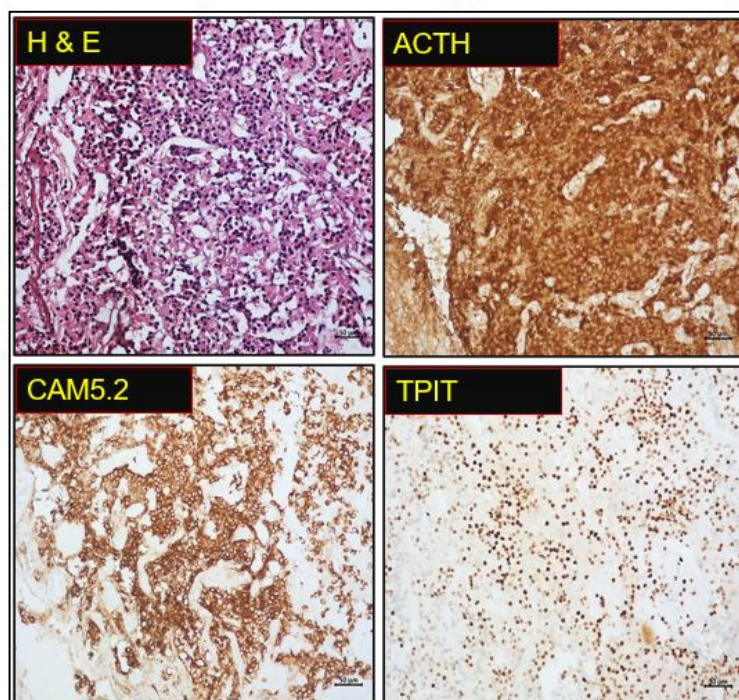


Figure 16: Case 3 - Sparsely granulated corticotroph tumor

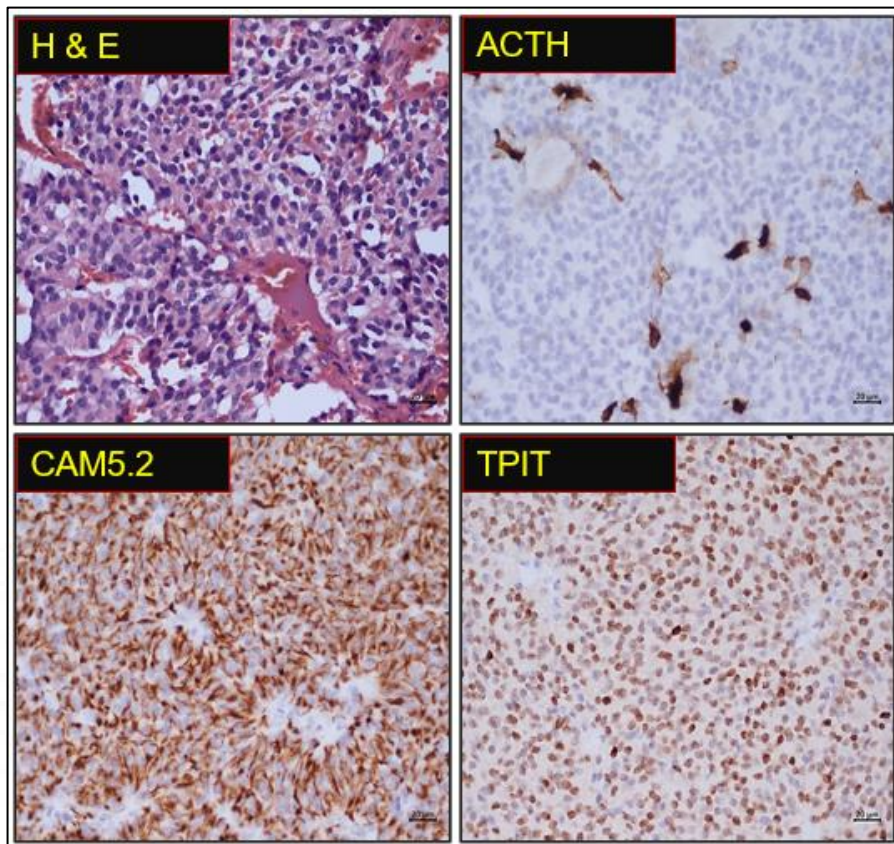
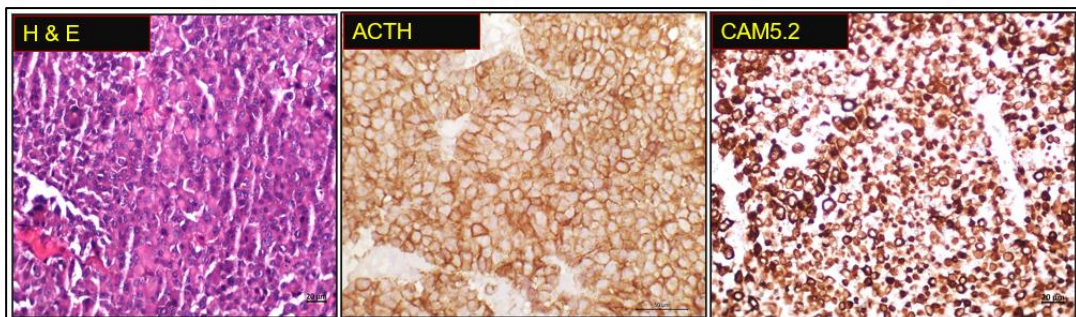


Figure 17: Case 4 - Crooke cell tumor



PIT1 Lineage:

Figure 18: Case 5 - Densely granulated somatotroph tumor

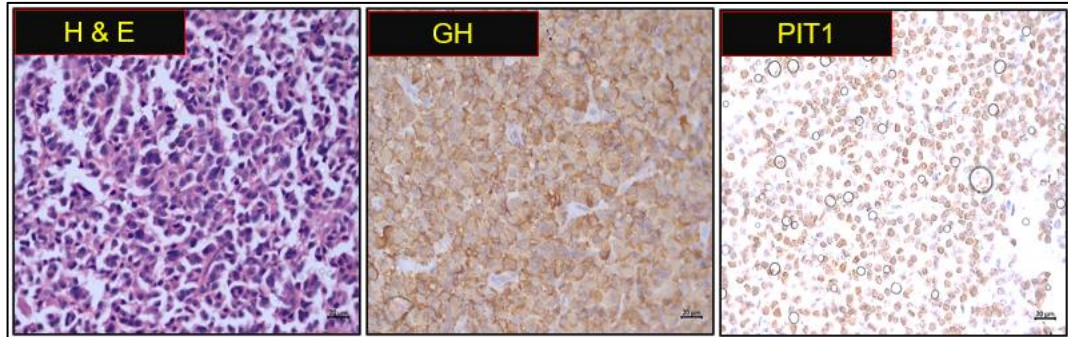


Figure 19: Case 6 - Sparsely granulated somatotroph tumor

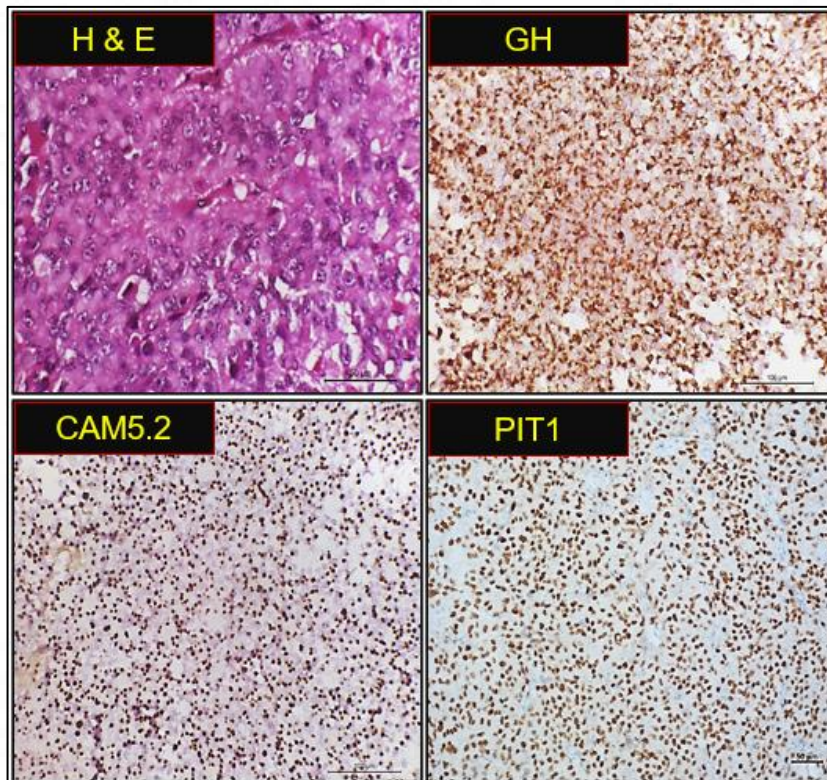


Figure 20: Case 7 - Mammosomatotroph PitNET/adenoma

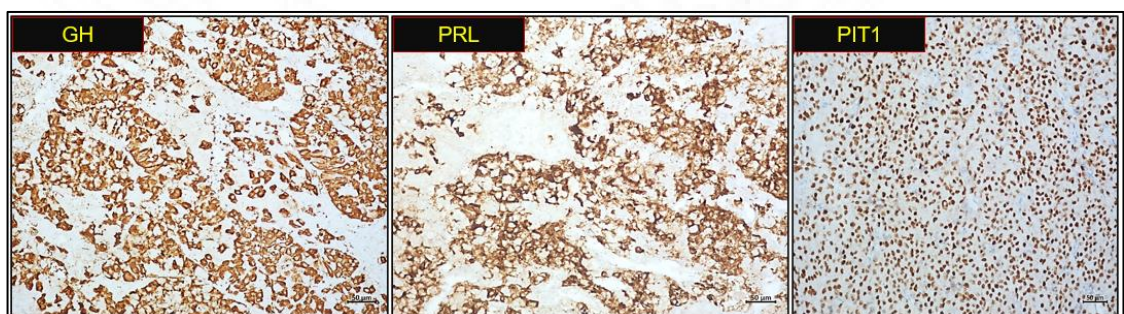


Figure 21: Case 8 - Densely granulated lactotroph tumor

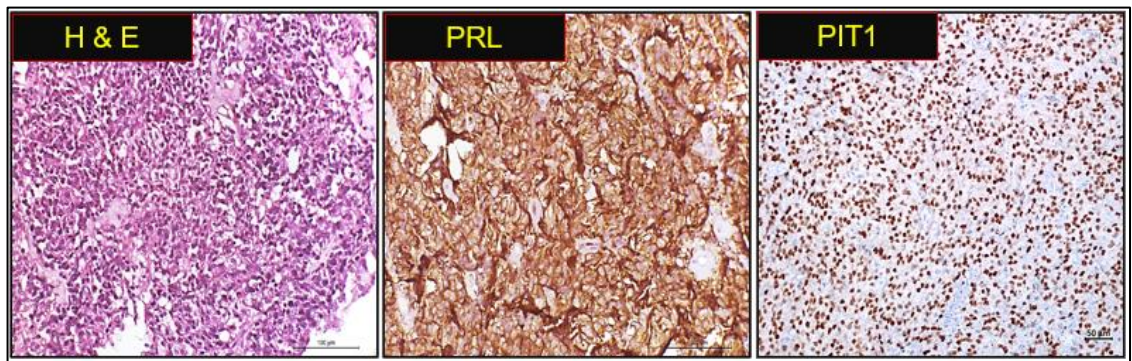


Figure 22: Case 9 - Sparsely granulated lactotroph tumor

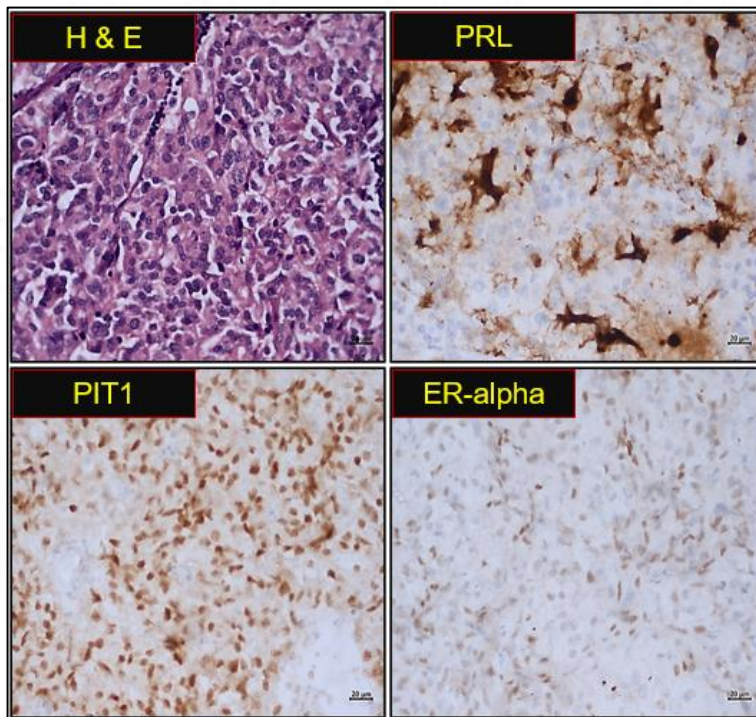


Figure 23: Case 10 - Mature plurihormonal PIT1-lineage PitNET/adenoma

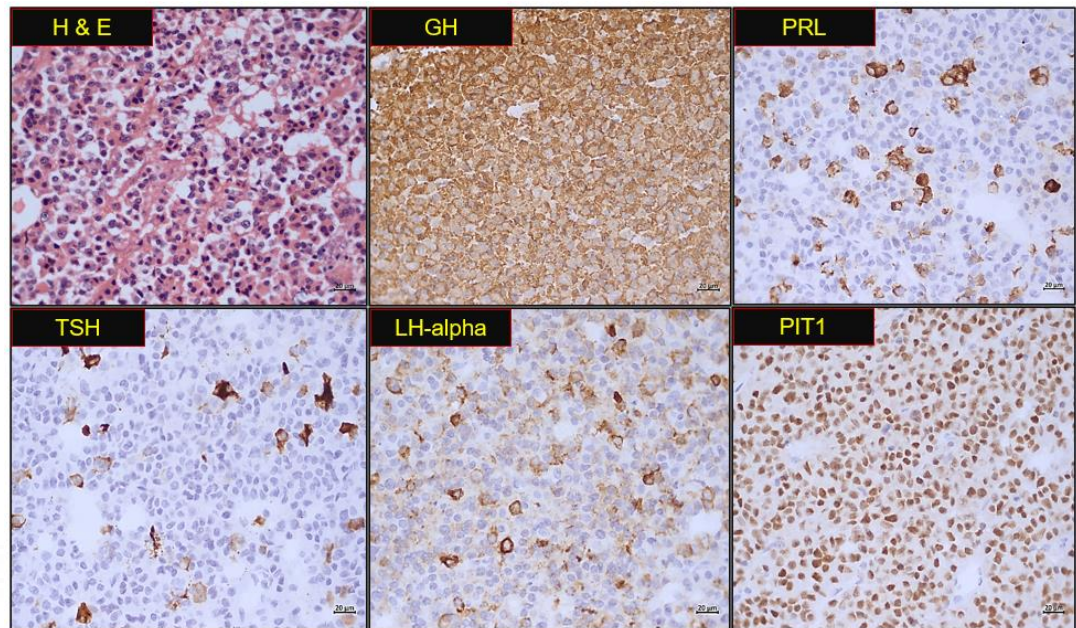
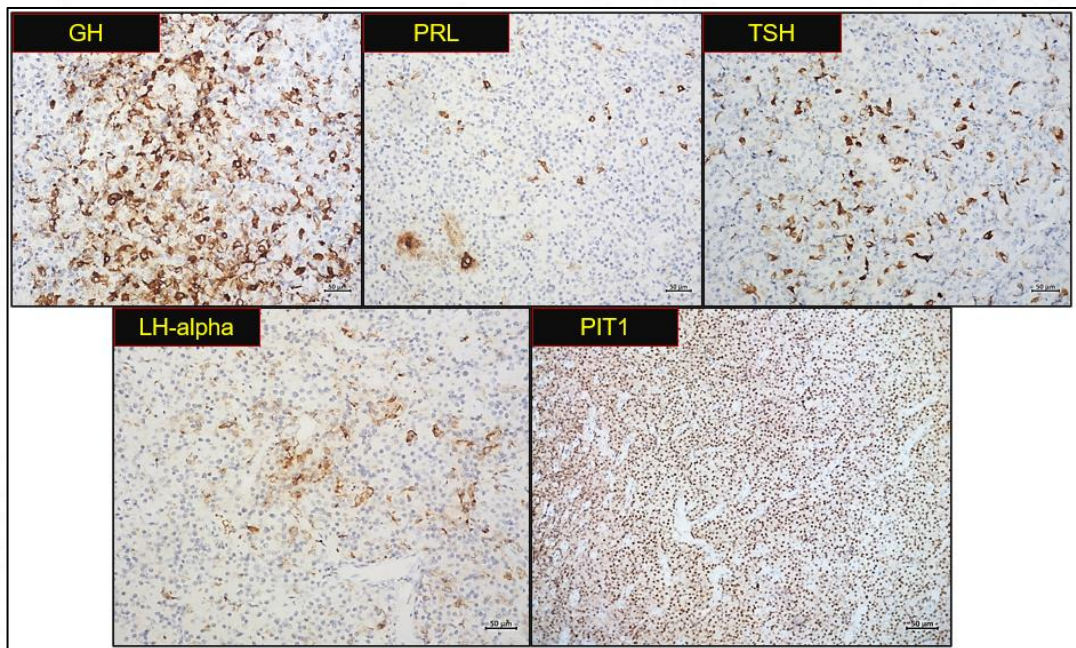


Figure 24: Case 11 - Immature PIT1-lineage PitNET/adenoma



PitNETs without distinct lineage differentiation:

Figure 25: Case 12 - Null cell PitNET/adenoma

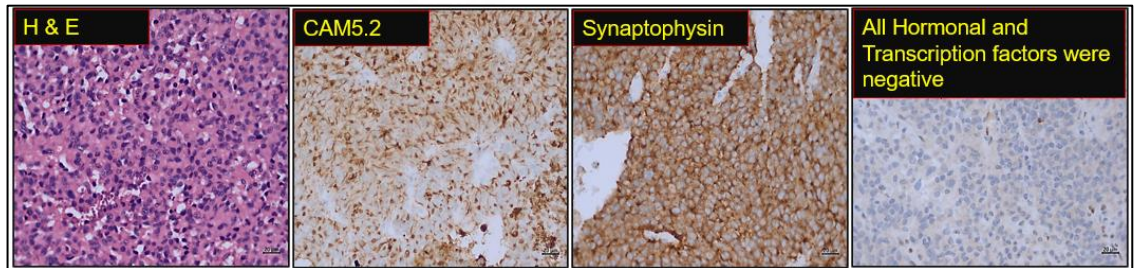
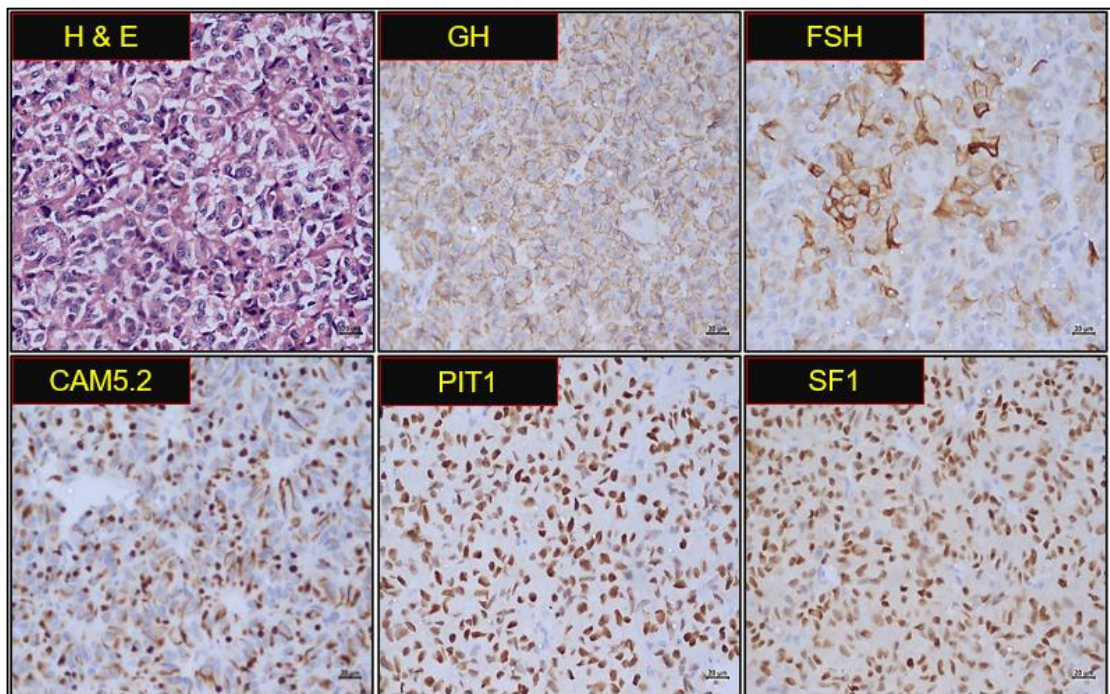


Figure 26: Case 13 - Plurihormonal PitNET/adenomas co-expressing PIT1/SF1



Multiple PitNETs:

Figure 27: Case 14 - Multiple synchronous PitNET/adenomas of distinct lineages

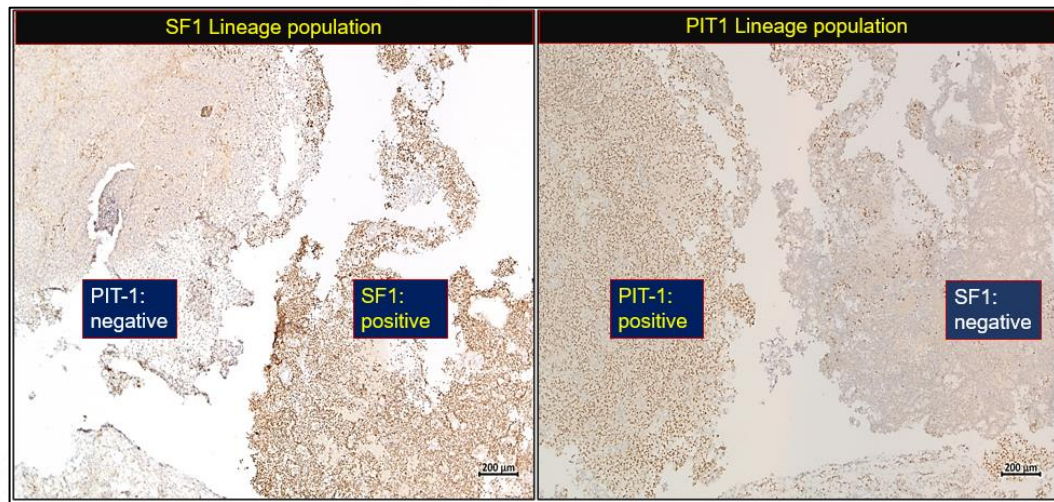
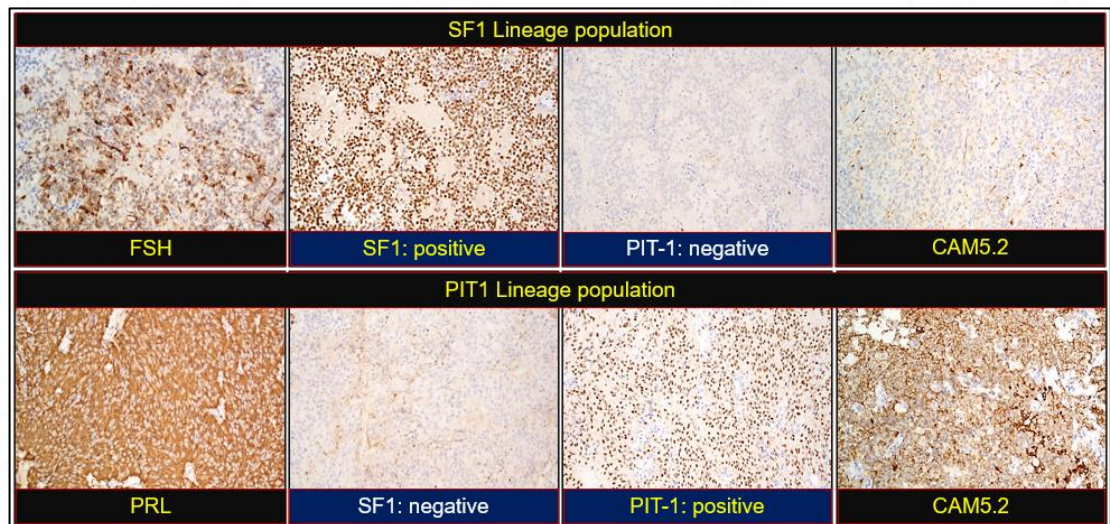
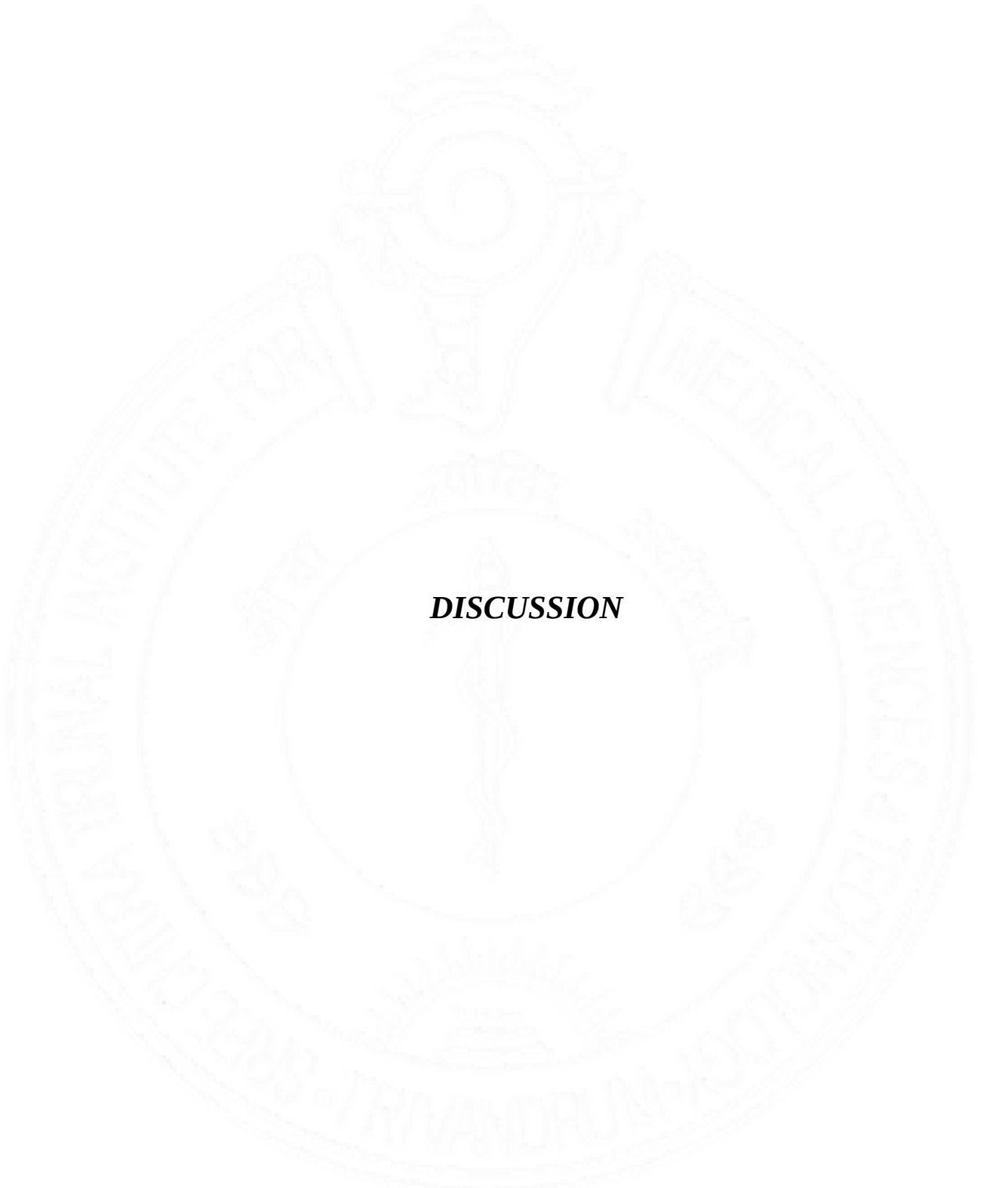


Figure 28: Case 15- Multiple synchronous PitNET/adenomas of distinct lineages





DISCUSSION

This study uniquely characterizes PitNETs as defined by the 2022 WHO classification, emphasizing the relevance of this classification system to clinical practice and patient outcomes. Prior work has established the importance of specific transcription factors, including SF-1, TPIT, and Pit-1 in PitNET classification since the 2017 WHO update (4,12,13,72–74). Our findings underscore the value of these markers for improved diagnostic accuracy and prognostic prediction, surpassing the limitations of previous classifications based on anterior pituitary hormone IHC (50,55).

Tumor Classification:

In this study, SF1 lineage was the predominant type followed by TPIT lineage, comparable to the research findings from Woo et al. and Kim et al. with significant association between lineage and patient age, sex (as in study by Kim et al.) and clinical presentation (12,13).

PIT1/SF1 co-expressing tumors constituted 4.8% of total cases, compared to 2.5% in study by Asa et al. (75).

Demographic data:

The Table given below shows a comparison of the demographic characteristics of PitNET patients across different studies (4,13,76,77).

Table No 15: Comparison of demographic data of PitNET with other published data

Demographic data	Present study (n=514)	LIU, J. et al (n= 250)	Kim et al (n=125)	Asmaro et al (n=169)	Woo et al (n= 113)
Mean Age(years)	46.54±13.418	51.71±12.87	57.6 ± 13.1	52	-
Sex	Males=Females (50% each)	Females (57.6%)	Females (50.4%)	Females (54%)	Males (48.7%)

Age distribution across lineages:

Our findings revealed a significant correlation between PitNET lineage and patient age. Younger patients (<30 years) were more likely to have PIT1 lineage and older patients (>45 years) SF1 lineage tumors ($p<0.001$). This was in alignment with findings by Woo et al., where mean age of SF1 lineage was 58.0 ± 12.2 years and PIT1 lineage was 41.9 ± 16.8 with a significant association between age-group and lineage ($p=0.006$) (12).

Gender difference across lineages:

Significant gender differences were noted among lineages. SF1 and PIT1 lineage PitNETs occurred significantly more commonly in males while TPIT lineage in females ($p<0.001$). A similar finding was noted in a study conducted by Kim et al., where TPIT lineage was predominantly observed in females ($p<0.001$), and SF-1 lineage was predominantly observed in males ($p<0.001$) (13).

Clinical profile of the patients:

Most common clinical manifestation was pressure symptoms presenting as

visual disturbance and headache, as in study by Liu J. et al (4). The other frequent presentation was endocrine symptoms such as acromegaly and Cushing disease. The clinical profile varied significantly between lineages with pressure symptoms being the most common presentation in SF1, TPIT and other PitNETs, while majority of PIT1 lineage tumors had both acromegaly and pressure symptoms ($p < 0.001$). Whereas in study by Liu J. et al, all the lineages commonly presented with pressure symptoms with no significant difference(4).

Incidence of apoplexy in PitNETs is 8.4% in the study, whereas an incidence rate of 2% was observed in study by Falhammar, H. et al (78).

Tumor size distribution of cases:

Giant adenomas were mostly of TPIT lineage, while most of the macroadenomas were SF1 lineage tumors and most of microadenomas were TPIT lineage tumors ($p < 0.001$). The study by Woo et al. did not find any significant association between PitNET lineage and tumor size, but there was a suggestion of a trend with a p-value of 0.066 (12). In addition, Woo et al, have taken the tumor volume from MRI for comparison, while, in this study, the tumor size has been used for comparison and the sample size is much smaller (113 vs 514).

Invasiveness across lineages:

The overall rate of cavernous or sphenoid sinus invasion in our study was 36.2% which was almost similar to those reported by Kim et al (32%) and Asmaro et al (33%). However, the latter studies have considered only cavernous sinus invasion, whereas, in our study both cavernous sinus and sphenoid sinus invasion were considered. In the present series, invasion of cavernous or sphenoid sinus was

significantly associated with TPIT and SF1 ($p < 0.001$) lineage tumors in our Study. In contrast, Kim et al. and Asmaro et al. reported that Pit-1 lineage tumors exhibited significantly greater cavernous sinus invasion compared to TPIT and SF-1 lineages with p value of 0.040 and <0.001 respectively (13,77). The percentage of tumors with sinus invasion in our study was: 28.5% (53/211) - SF1, 43% (80/172) - TPIT and 44.7% (42/94) – PIT1, while in the study by Kim et al, it was: 16.9% (12/71) - SF1, 50% (14/28) - TPIT and 58.3% (7/12) - PIT1 and Asmaro et al, it was: 18% (11/62) - SF1, 24% (9/38) - TPIT and 53% (35/64) - PIT1. These numbers are relatively similar between our study and that of Kim et al, when one takes into consideration the sample size differences between the two series (514 vs 113). In the study by Asmaro, et al, PIT1 lineage tumors were over-represented and TPIT tumors were under-represented, while in our series, it was vice-versa which may account for some of the differences in lineage-related invasion of cavernous sinus. However, the reason for higher invasiveness of SF1 lineage tumors in our series when compared to that of Asmaro,et al, is still not clear.

Prognostic factors:

Statistically significant differences in median progression-free survival (PFS) were found across PitNET lineages, as shown by Kaplan-Meier analysis, with shorter PFS for TPIT and SF1 lineages (log-rank $p=0.039$), invasive tumors ($p=0.019$), and higher tumor grades – worst for Grade 2b ($p=0.012$). Kim et al. found, through Kaplan-Meier analysis, that progression-free survival (PFS) differed significantly among lineages, with Pit-1 showing a higher rate of progression and recurrence than TPIT ($p = 0.004$). Considering the differences in sample size

between the two series, it is not possible to draw logical conclusions regarding the reasons for differences in progression-free survival between the present series and that of Kim, et al. (13).

In Cox univariate analysis, significantly higher progression/ recurrence rates were noted across TPIT ($p=0.005$) and SF1 (0.050) lineages, invasive tumors ($p=0.021$), and higher-grade tumors: Grade 2b ($p=0.014$). However, Cox multivariate analysis identified the PitNET lineage as the sole independent predictor of progression/recurrence in PitNETs, with TPIT and SF1 lineages significantly associated with tumor progression/recurrence ($p=0.007$). Thus, invasiveness, higher tumor grade and TPIT lineage were associated with worse outcome. Kim et al. found that cavernous sinus invasion and high-risk PAs were associated with a significant increase in progression and recurrence rates using Cox multivariate analysis (13). However, in their multivariate analysis, Kim et al, did not include PitNET lineage as a variable, but only included the five aggressive subtypes as high-risk group and the remaining subtypes as low-risk group. In our study, we did not include the high-risk subtype as a variable.

Studies by Raverot et al. (2017), Lelotte et al. (2018), and Asioli et al. (2019) demonstrated that higher tumor grade, based on a five-tier system, was a strong predictor of post-operative disease-free outcome or recurrence or progression-free status, with grade 2b showing the poorest outcome (7,54,59,60). However, in our study, the tumor grade did not emerge as an independent prognostic factor for recurrence or progression-free survival in Cox multivariate survival analysis ($p=0.777$).

Limitations of 2022 WHO classification:

Lineage-based classification advancements in the 2017 and subsequently in 2022 WHO classifications have enhanced our knowledge of pituitary tumor characteristics and suggest potential improvements in predicting outcomes. But its limitations include:

- Lack of clear diagnostic cut-offs for transcription factor immunopositivity.
- Variability in laboratory techniques and IHC processes (52,72).
- Significant financial and logistical barriers to widespread adoption of transcription factor analysis in clinical laboratories (52).
- The detection of recommended transcription factors is hindered by the scarce and unreliable commercial availability of suitable antibodies (72).
- The 2022 WHO classification have not adequately addressed the integration of various prognostic factors, including clinical, radiological, and pathological features. No grading system is in place for risk stratification.
- The 2022 WHO classification does not provide clear guidelines on the use of markers of proliferation like Ki67 index and mitotic count, due to the absence of standardized methods and clear cut-offs.

Future directions in PA classification:

Future research should focus on developing a comprehensive prognostic model that integrates multiple clinical, radiological, and pathological factors, including lineage-specific biomarkers.

Careful selection and proper use of antibodies are essential for accurate assessment of IHC marker positivity, which leads to reliable diagnoses. According to a study by Lenders et al., 10% threshold may be appropriate when other transcription factors are not expressed but require further validation (52). Automated image analysis algorithms hold potential for enhancing the accuracy and reproducibility of immunohistochemical assessments, although further research is needed in this area (Asa et al., 2022) (11).

Landers et al. recently suggested a tiered method for applying the 2022 WHO classification, which resulted in complete diagnostic agreement and a 34% cost reduction compared to a full assessment involving all transcription factors and hormone immunohistochemistry (79).

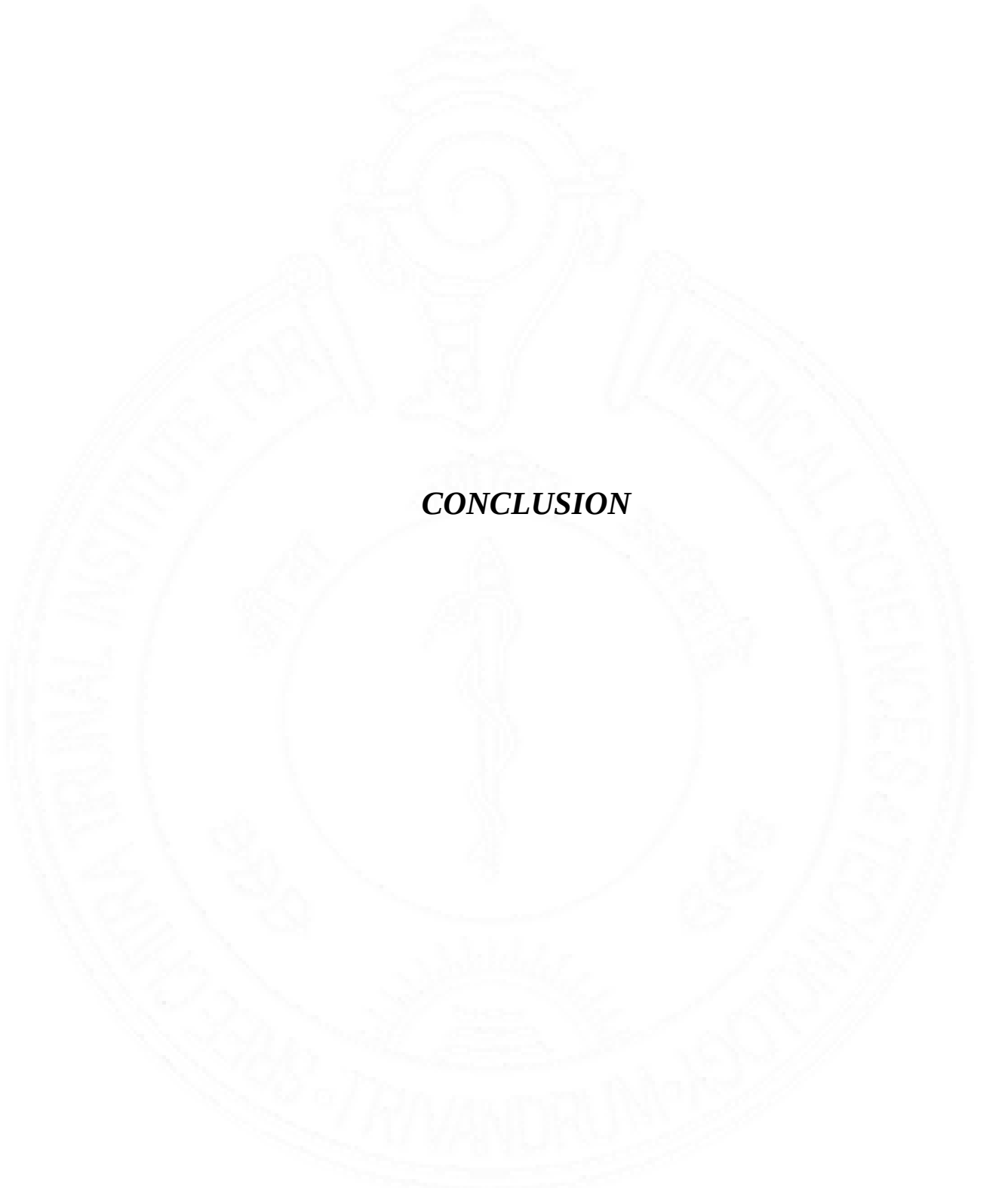
A grading system combining radiological assessment of tumor spread with detailed cell cycle proliferation markers was proposed by Trouillas et al.(54). This integrated approach, which has been validated in multiple studies, correlates with with long-term patient outcomes free from disease and progression (7,59,60). However, this classification does not adequately account for the substantial variability in clinical outcomes observed across different tumor lineages, as highlighted by recent studies and the 2022 WHO classification (54). Continued research and collaboration are essential to overcome these challenges and improve

the diagnosis and management of pituitary tumors.

Emerging molecular markers, including PitNET miRNA expression profiles, DNA methylation, and VEGF-A/VEGFR expression, hold promise for assessing tumor invasiveness and aggressiveness, though further research is needed due to limited availability (80).

Limitations of our study

While our study demonstrated significant variations in disease-free survival among PitNET subtypes, there are inherent limitations. The study's retrospective methodology and reliance on stored surgical samples, potentially affecting IHC staining, should be taken into account. The relatively short follow-up period (mean 27.56 ± 26.25 months) also limits the scope of our analysis regarding long-term outcomes. Future studies incorporating prospective data collection, fresh tissue samples, and extended follow-up are crucial for confirming our findings and identifying additional predictors of aggressive behavior.



CONCLUSION

This study underscores the clinical and pathological heterogeneity of Pituitary Neuroendocrine Tumors (PitNETs) in South India, aligned with the 2022 WHO classification. We identified significant associations between clinical factors, PitNET lineages, and patient outcomes. Our key findings:

- In our cohort of 514 PitNETs, SF1 lineage tumors were the most prevalent, followed by TPIT lineage tumors.
- SF1 lineage was more common in males and TPIT lineage in females.
- Younger patients were more likely to have PIT1 lineage and older patients SF1 lineage tumors.
- SF1 and TPIT lineages primarily presented with pressure symptoms, while PIT1 lineage tumors often presented with both pressure and endocrine symptoms.
- Giant adenomas were mostly TPIT lineage, while most of the macroadenomas were SF1 lineage tumors.
- Invasion was more common in TPIT and SF1 lineage tumors.
- Sparsely granulated corticotroph, mature plurihormonal PIT1, sparsely granulated somatotroph and sparsely granulated lactotroph subtypes were associated with higher grades.
- TPIT lineage, invasive tumors, and higher-grade tumors were associated with worse progression-free survival.



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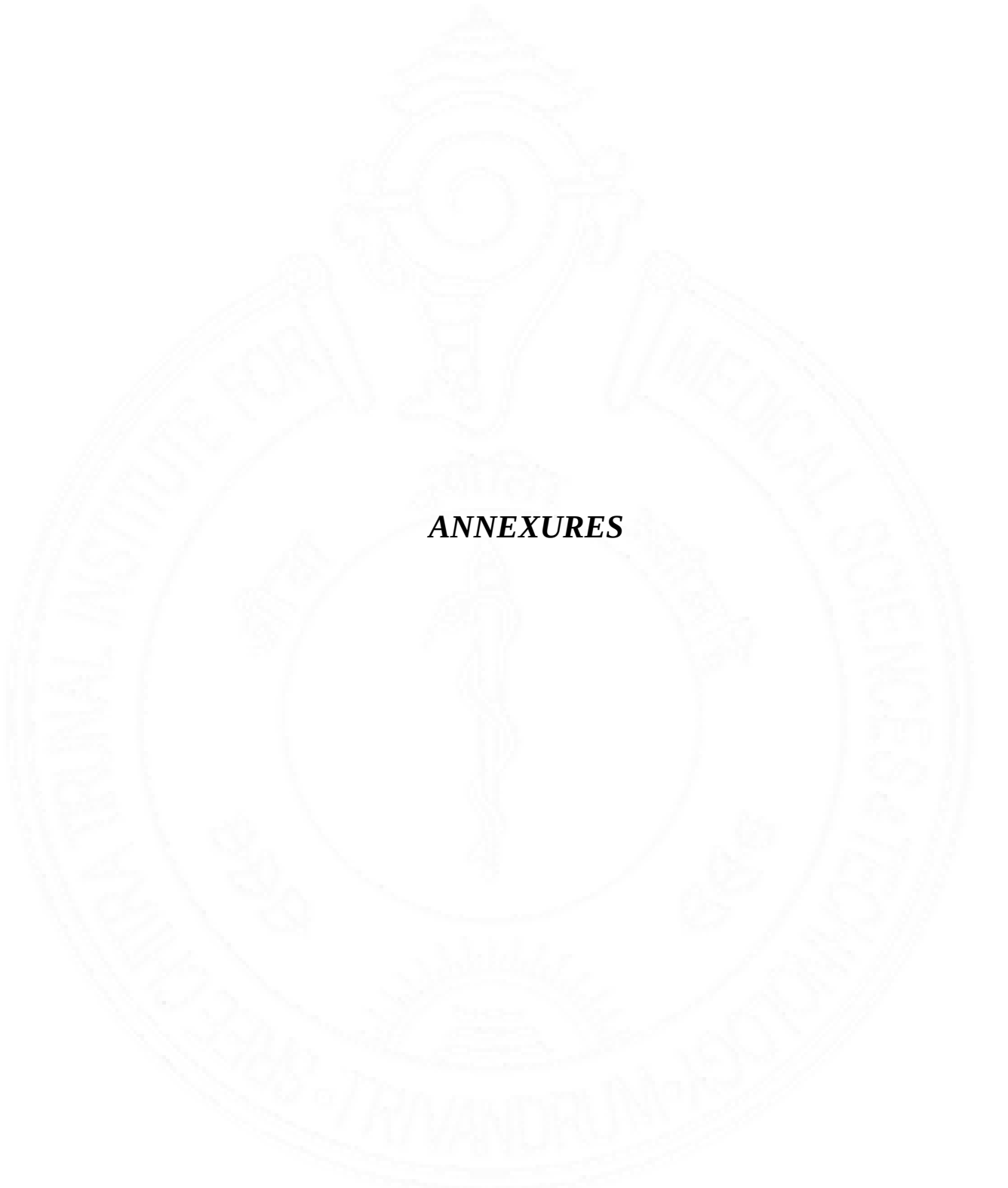
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ANNEXURES



श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
तिरुवनन्तपुरम - ६९५०११, केरल, इंडिया

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Institutional Ethics Committee

CDSO Registration No: ECR/189/Inst/KL/2013/RR-21
DHR Registration No: EC/NEW/INST/2022/2775

SCT/IEC/2205/MARCH/2024

19.04.2024

Dr. Silvie Thomas
PDCC in Neuropathology
Department of Pathology
SCTIMST, Thiruvananthapuram

Dear Dr. Silvie Thomas,

The Institutional Ethics Committee held on 16th March, 2024, reviewed and discussed your application to conduct the study titled "SPECTRUM OF PITUITARY NEUROENDOCRINE TUMORS (PITNETS) IN A TERTIARY CARE CENTRE: CLASSIFICATION BASED ON 2022 WORLD HEALTH ORGANIZATION GUIDELINES (IEC /2205) "

Principal Investigator	Dr. Silvie Thomas, Department of Pathology, SCTIMST
Co-Principal Investigator(s)	Dr. Deepti AN, Department of Pathology, SCTIMST Dr. Rajalakshmi P, Department of Pathology, SCTIMST
Co- Investigator(s)	Dr. Prakash Nair, Department of Neurosurgery, SCTIMST Dr. Bejoy Thomas, Department of Imaging Sciences and Interventional Radiology, SCTIMST
Duration of the study	12 months

The following members of the Ethics Committee were present at the meeting held on 16th March, 2024

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Prof. C.C. Kartha	MBBS,MD	Male	Basic Medical Scientist (Chairman)	No
2.	Dr. Pradeep S	MBBS, MD	Male	Basic Medical Scientist	No
3.	Smt. Sathi Nair	MA (English Literature)	Female	Lay Person	No
4.	Adv. N Anand	BAL, L.LB	Male	Legal Expert	No
5.	Dr. P. Manickam	BSMS, MSc (Epid).,PhD	Male	Health Science Expert/ Social Scientist	No
6.	Dr. Christina George	MD Psychiatry	Female	Clinician	No
7.	Dr. Harikrishna Varma PR	Ph.D (Materials Science)	Male	Medical Technology	Yes
8.	Dr. Manikandan.S	MBBS,MD,PDCC	Male	Clinician	Yes
9.	Dr. Narayanan Namboodiri. K.K	MBBS,MD,DM	Male	Clinician	Yes
10.	Dr. Biju Soman	MBBS,MD, DPH, MSc, DLSHTM	Male	Basic Medical Scientist	Yes
11.	Dr. Syam K	MBBS, MD, DM	Male	Clinician	Yes
12.	Dr. Srinivas G	PhD	Male	Basic Medical Scientist (Member Secretary)	Yes

The following documents were reviewed:Original submission

1. Covering letter addressed to the Chairperson, IEC, SCTIMST dated 15.01.2024
2. Checklist Form
3. IEC Application Form
4. Declaration Form
5. Study Proposal
6. Proforma
7. CV of PI and Co-PIs
8. Copy of TCMC Registration forms
9. SRC Recommendation Letter

Revised submission

1. Covering letter addressed to the Chairperson, IEC, SCTIMST dated 18.04.2024
2. Checklist Form
3. IEC Application Form
4. Declaration Form
5. Study Proposal
6. Proforma
7. CV of PI and Co-PIs
8. Copy of TCMC Registration forms

IEC Decision

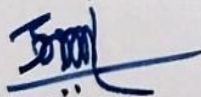
The IEC approved the conduct of the study in the present form.

Remarks:

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,



Dr. G. Srinivas
Member Secretary, IEC

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE (IEC)
SCTIMST, THIRUVANANTHAPURAM



PLAGIARISM CHECK REPORT

3/1/25, 9:34 AM

Similarity Report

Silvie thesis

By: Deepti A N

As of: Feb 28, 2025 10:31:32 PM
8,260 words - 23 matches - 3 sources

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paper text:

INTRODUCTION Pituitary adenomas represent a spectrum of neoplasms originating in the anterior pituitary gland. They represent a significant proportion of intracranial neoplasms (1,2). The patients can be asymptomatic or can present with hormone over secretion associated symptoms or pressure symptoms (3,4). Though initially considered benign, these tumors showcase diverse clinical behaviors, aggressiveness and high rate of recurrence (5). The previous 2017 WHO classification introduced pituitary adenoma classification based on immunohistochemistry (IHC) for transcription factors and hormonal markers and recognized some subtypes as "high-risk pituitary adenomas(6)". It has served as a foundation for numerous studies exploring the clinical and pathological features of pituitary adenomas, underscoring the value of immunohistochemical profiling for predicting tumor outcomes (7,8). Acknowledging the aggressive potential, the recent 2022 World Health Organization (WHO) classification system introduced the nomenclature "Pituitary Neuroendocrine Tumors (PitNET)", replacing the term "adenoma". It put forward significant revisions with an emphasis on detailed histological subtyping of PitNETs based on the tumor cell lineage, cell type, and related characteristics according to combined immunohistochemical expression of pituitary hormones (GH, PRL, beta-TSH, beta-FSH, beta-LH, ACTH and alpha-subunit), transcription factors (PIT1, TPIT, SF1, GATA2/3 and ER-alpha) and keratin (CAM 5.2) (9-11). Thus, the PitNETs are currently categorized into four distinct groups: PIT-1-lineage PitNETs, TPIT-lineage PitNETs, SF-1-lineage PitNET, and PitNETs with no distinct lineage 3 differentiation (9,11). While precise tumor classification is crucial, the assessment of proliferation using mitotic count and Ki-67 index, and radiological or histological evidence of tumor invasiveness have prognostic significance while identifying possible aggressive pituitary adenomas (7,8). The full clinical and patient outcome implications of the revised 2022 WHO classification for PitNETs is yet to be fully determined. Emerging research has begun to explore the clinical significance of this updated WHO classification system, demonstrating its potential to improve prognostic accuracy and inform treatment strategies. These studies have shown promising correlations between specific PitNET subtypes defined by the 2022 WHO classification and clinical aggressiveness, recurrence rates, and treatment response (12,13). While existing studies on PitNETs utilizing the 2022 WHO classification have shown promise, most have been conducted in relatively small patient groups and require validation in larger and more diverse populations. Moreover, the most effective treatment approaches for each PitNET subtype defined by the 2022 WHO classification remain an area of active investigation.

Our study aims to contribute to the

1

ongoing research by conducting a comprehensive analysis of PitNETs in a South Indian population, utilizing the 2022 WHO classification. Specifically, this study seeks

PROFORMA

Case study no:

1. Age:
2. Gender:
3. Clinical manifestations: pressure symptoms/ hormonal- functional/non-functional
4. Radiological- tumor size, invasion, density on T2 weighted imaging
6. Invasive/non-invasive
7. Presurgery treatment
8. Histopathology: diagnosis/grading/invasion/apoplexy
9. Immunohistochemistry:

Cytokeratin- CAM 5.2 - positive/ negative

Lineage - specific transcription factors (PIT1, TPIT, ER-alpha, GATA2, SF1) - positive/
negative

Hormonal markers- (GH (Growth Hormone), PRL(Prolactin), ACTH, TSH-beta, FSH-beta, LH-
beta, LH-alpha)- positive/ negative

p53 - positive/ negative,

Proliferation marker (Ki-67)- in percentage

10. Follow up-

Follow-up (months):

Recurrence: Present/Absent

If present, time to recurrence (months):

Progression: Present/Absent

If present, time to progression (months):