SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

Thiruvananthapuram – 695011

PROJECT REPORT

Name: *Dr. Anudath.K.B*

Programme: *M.Ch. NEUROSURGERY*

Month and Year of Submission: *NOVEMBER - 2001*

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

THIRUVANANTHAPURAM

Project Report

Title of the Project:

"Nonfunctional Pituitary Adenoma - Clinical & Immunohistochemical Analysis"

Name: **Dr. Anudath.K.B.**

Programme: **MCh Neurosurgery**

Month & Year of Submission: November 2001

CERTIFICATE

I, Dr. Anudath.K.B hereby declare that I have actually carried out the project **"Nonfunctional Pituitary Adenoma - Clinical & Immunohistochemical Analysis"** independently under supervision and guidance in the institution.

Thiruvananthapuram, Signature .....

Date: 24 October 2001 Name: **Dr. Anudath.K.B.**

Forwarded.

He has carried out the above-mentioned project in the department of Neurosurgery, SCTIMST, Thiruvananthapuram.

Signature .....

Prof. R.N. Bhattacharya

Head of the department of Neurosurgery,

SCTIMST, Thiruvananthapuram.

Contents

Acknowledgement

This work has involved the inspiration, support and co-operation of many. Foremost among them was that of Prof. R.N. Bhattacharya, professor and head of the department of neurosurgery, who had taken a personal interest in the study. At the outset, let me express my heart felt respect and gratitude towards him for his invaluable guidance, constant review and keen interest in the ongoing work

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I am grateful to my parents for their silent encouragement and support. Finally I must put on record my indebtedness and love for my wife Archana, who in the last 3 years has coped with me and for all her active support in this endeavor.

Dr. Anudath.K.B


Introduction

Pituitary Adenoma

Pituitary adenomas are one of the common intracranial neoplasms in adults. They compose approximately 10% of diagnosed brain tumors. Pituitary tumors have been categorized by the hormones they secrete, the resulting syndromes of clinical hormone excess, and the pituitary cell type of origin.

Histologically, pituitary tumors are classified according to their tinctoral staining characteristics (acidophilic, eosinophilic, chromophobe adenomas), but these analyses correlate only loosely with type of hormone produced. In recent years, immunohistochemical studies, using antibodies specific for each of the major pituitary hormones, have been used to define tumor phenotype.

Immunohistochemical techniques and the use of specific antisera to directly identify the hormones stored within the cells was a turning point in the morphological study of pituitary adenomas. This led to the finding that many "nonfunctioning" adenomas contained one or more pituitary hormones, even though their presence did not result in an increased blood concentration of pituitary hormones. All pituitary hormones have been found in nonfunctioning tumors, but according to different investigators the most frequently present molecules are gycoprotein hormones and / or their uncombined α and β subunits.

Endocrine-inactive pituitary adenomas are considered to be of gonadotroph cell origin because, although they do not cause a recognized syndrome of excess hormone over-production, they often secrete intact gonadotropins and /or their free subunits.[1,2] Such tumors are slow-growing macroadenomas with extrasellar extension, and patients often present with symptoms of mass effect, including headache, visual field deficits, cranial nerve palsies, and associated hypopituitarism. Nonfunctioning Pituitary adenomas account for approximately 30% of Pituitary tumors.

NULL CELL PITUITARY TUMORS

Null cell adenomas, or clinically nonfunctioning tumors, are variably defined depending upon the criteria used to analyze tumor cell phenotype. As noted above, the majority of clinically nonfunctioning adenomas can be shown to produce low levels of the free a-subunit, FSH, and, to a lesser degree, LH when analyzed by immunocytochemistry or for mRNA expression. A smaller fraction can be shown to produce low levels of other pituitary hormones, particularly ACTH or GH, which escaped detection by routine endocrine testing. Even with detailed analyses of hormone production, a subset (10 to 20%) of nonfunctioning adenomas does not appear to produce one of the major pituitary hormones.

This study aims at analyzing nonfunctional pituitary adenoma cases with respect to their presentation and by immunohistochemistry using a series of antibodies raised against different hormones to detect hormone production within these tumors. This would help to further define the cellular phenotype of pituitary adenomas and in characterizing the natural history of different subtypes of these tumors. Also it could be used in selecting patients for adjuvant medical therapies directed specifically against different tumor types.


Review of
Literature

Pituitary tumour

Pituitary adenomas account for about 10-20 per cent of intracranial neoplasms and occur more commonly in women. They reach a peak incidence between the third and sixth decades of life. Microadenomas are distinguished from adenomas solely on the basis of size; the formers are less than 10mm in diameter. Micro-adenomas come to attention when they produce endocrinologically active substances. Macroadenomas are often nonsecretory and are discovered because of the mass effect they exert.

All pituitary adenomas appear to arise from one pituitary cell type to another - lactotroph, somatotroph, corticotroph, thyrotroph or gonadotroph - identifying some types of adenomas is more difficult than others. Identifying an adenoma that secretes a relatively small amount of hormone or a hormone that doesn't produce a recognizable clinical syndrome is more difficult. These adenomas can be called "clinically nonfunctioning", because of the lack of clinical syndrome attributable to hormone function.

Any kind of pituitary adenoma can be clinically nonfunctioning, but some types are clinically nonfunctioning usually and other types are clinically nonfunctioning unusually. Gonadotroph adenomas, for example are usually clinically nonfunctioning for two reasons. First, they usually do not secrete as efficiently as does the normal gonadotroph cell and they do not usually secrete both intact FSH and LH. Second, even when they secrete enough FSH to raise the serum FSH concentration above normal, a supranormal serum FSH concentration usually does not produce a recognizable clinical syndrome, especially in men or post-menopausal women. Thyrotroph adenomas are also usually clinically nonfunctioning, because they usually do not produce intact TSH. Those that do, of course, cause hyperthyroidism. Lactotroph adenomas do produce a recognizable clinical syndrome in premenopausal women - oligomenorrhea or amenorrhea and less often, galactorrhea - but not in postmenopausal women who are already amenorrheic. Lactotroph adenomas in men

often cause symptomatic hypogonadism and impotence, but the cause of these symptoms may not be readily recognized.

At the other end of the spectrum are somatotroph and corticotroph adenomas, which usually produce recognizable clinical syndromes, acromegaly and Cushing's syndrome, respectively. Even somatotroph and corticotroph adenomas, however, may be clinically nonfunctioning. Several cases have been reported in which the adenomas were identified immunocytochemically but the patients did not, even on reexamination, have the usual clinical syndrome or even elevated serum concentrations of growth hormone or corticotrophin (ACTH). [3,4,5,6,7]

Immunohistochemical techniques differentiate the individual subtypes and allow a classification to be made according to the tumor's endocrine status.

Approximately 30% of the pituitary adenomas are currently thought to be clinically nonfunctioning.[8,9,10,11,12] 23%,[2] out of 160 patients in study by Peter Black. Based on immunocytochemical analyses for detecting PRL, ACTH, GH, LH, FSH and TSH, different surgical series have reported the percentage of adenomas without apparent products as 25.5 %,[11] 34%,[13] 40%.[10] and 16.7%[14]

Kovacs, et al.,[15] De Marco, et al.,[16] and Esiri, et al.,[10] have all reported scattered staining for a variety of hormone products in their clinically silent tumors. Esiri, et al., found this in 40% of cases.

Silent ACTH-secreting adenomas have been found in several other series.[17,13,18,19] Horvath, et al.[3], found that 4.3% of tumors staining for ACTH were clinically silent.

Clinically nonfunctioning adenomas presents with neurological symptoms or hormonal symptoms. The most common presentation is by one or more neurological symptoms –

1) impaired vision
2) headache
3) diplopia caused by oculomotor nerve compression in cavernous sinus
4) CSF rhinorrhea caused by inferior extension of adenoma eroding sella and
5) headache and diplopia caused by pituitary apoplexy.

The clinical presentation is determined by the extent of any spread. As the pituitary tumour expands, compression of adjacent structures follows. The type of visual defect produced depends on the relationship of the pituitary fossa to the optic chiasm. With the most common arrangement, compression of the chiasm from below produces a superior bitemporal field defect, which is typically asymmetric.

Hypopituitarism results either from compression of the gland by an endocrinologically inert tumour or acutely as a result of infarction of, or haemorrhage into, the gland (pituitary apoplexy). Typically, patients develop severe headache, ophthalmoplegia and signs of subarachnoid haemorrhage if the basic process is haemorrhagic.

CLASSIFICATION

Pituitary tumors are classified according to the hormones that they produce. Histologically, pituitary tumors are also classified according to their tinctoral staining characteristics (acidophilic, eosinophilic, chromophobe adenomas), but these analyses correlate only loosely with type of hormone produced. Immunohistochemical studies, using antibodies specific for each of the major pituitary hormones, have been used to define tumor phenotype. Electron microscopy can provide additional ultrastructural information but is not used routinely.

The World Health Organization Classification of Adenohypophysial neoplasms.[20]

Functional Classification of Adenohypophysial tumors

A. Endocrine hyper function
    1. Acromegaly/Gigantism, elevated growth hormone levels
    2. Hyperprolactinemia and sequelae
    3. Cushing's disease, elevated adrenocorticotropic hormone and cortisol levels.
    4. Hyperthyroidism with inappropriate hypersecretion of thyrotropin
    5. Significantly elevated follicle stimulating hormone and luteinizing hormone and / or alpha subunit
    6. Multiple hormonal overproduction

B. Clinically nonfunctioning

C. Functional status undetermined

D. Endocrine hyperfunction due to ectopic sources

1. Clinical acromegaly secondary to ectopic growth hormone-releasing hormone overproduction (hyperplasia/adenoma)

2. Cushing's disease secondary to ectopic corticotrophin-releasing hormone overproduction (hyperplasia/adenoma)

Immunohistochemical Classification of Adenohypophysial Tumors[20]

Principal Immunoreactivity	**Secondary Immunoreactivity**
A. GH	PRL, a-SU(f), TSH, FSH, LH(i)
B. PRL	a-SU(i)
C. GH and PRL	a-SU (f), TSH (i)
D. ACTH	LH, a-SU(i)
E. FSH/LH/a-SU	PRL, GH, ACTH (i)
F. TSH	a-SU, GH, ACTH(i)
G. Rare hormone combinations	
H. Immunonegative	

GH: Growth Hormone; PRL:Prolactin; a-SU: alpha-subunit; f: frequent; TSH: Thyrotropin; FSH: Follicle Stimulating Hormone; LH: Luteinizing hormone; I: Infrequent; ACTH: Adenocorticotropic hormone

Immunohistochemistry and the histological localization of the following hormones in pituitary tumours have been well demonstrated.

ACTH: The antibody labels the Corticotrophs in the adenohypophysis and is useful in the classification of pituitary adenoma.

FSH: This hormone of 35 KD, which is involved in the maturation of ovarian follicle and oestrogen secretion in females. Immunolabeled gonadotrophic cells are used in the classification of pituitary adenomas.

HGH: somatotroph cells produce this hormone. One of the effects of GH is to stimulate protein synthesis although the actual mechanism is not well understood .It is useful in the classification of pituitary adenomas.

TSH: It is of 28 KD, which stimulates thyroid growth and production of thyroid hormones. This antibody labels thyrotrophic cells and is used in the classification of pituitary adenomas.

Prolactin: It is of 23 KD involved in the stimulation of milk production, salt and water regulation growth development and reproduction. Prolactin producing cells make up approximately 20% of the cells of the normal pituitary.

Clinically nonfunctioning adenomas are not associated with any abnormalities of basal or stimulated concentrations of any pituitary hormones or their subunits. When these adenomas are studied in vitro, however, the cell of origin can usually be identified. Several different in vitro techniques have been used to identify the nature of pituitary adenomas, and the results have been similar whatever the technique. Using the different techniques, 70% to 100% of adenomas that could not be recognized in vivo have been found to synthesize or secrete some combination of intact FSH and LH and α, FSHβ, and LHβ subunits. Up to 25% of these adenomas demonstrate intact TSH, TSHβ, or α, but only sporadic adenomas show evidence of prolactin, growth hormone or ACTH.[21]

Pituitary hormones demonstrated in Clinically nonfunctioning pituitary adenomas

Authors	Black et al[2]	Asa et al[22]	Jameson et al[1]	Croue et al[23]	MM Esiri et al[10]
No. of patients	37	12	14	40	48
FSHβ, LHβ, α	66.7 %	100 %	78.6 %	37.5 %	20.8 %
TSHβ, α	33.3 %	25 %	28.6 %	2.5 %	2.1 %
Prolactin	16.7 %	0	7.1 %	0	14.6 %
GH	2.8 %	0	7.1 %	0	2.1 %
ACTH	8.5 %	0	7.1 %	10 %	12.5 %

MASS EFFECTS OF PITUITARY ADENOMAS. Many of the clinical manifestations of pituitary adenomas are related to the hypersecretion of hormones. However, the mass effects of the enlarging tumor can also lead to specific signs and symptoms. Particularly in the case of nonfunctioning tumors or those that produce gonadotropins, the primary clinical manifestations are related to effects of the tumor on surrounding structures.

Headaches are common in patients with macroadenomas and appear to be caused by expansion of the diaphragma sellae or by invasion of bone. Headaches may be retro-orbital or referred to the top of the skull, but the location is variable. Severe headache associated with nausea, vomiting, and altered consciousness can also be caused by infarction of a pituitary adenoma. In severe cases, pituitary apoplexy can occur and may require urgent surgical decompression.

The effects of pituitary tumors on the visual fields are well explained by the relationship of the optic chiasm to the sella turcica. Expansion of macroadenomas into the suprasellar region exerts pressure on the optic chiasm, usually in the central region where nerves emanating from the inferior and medial part of the retina (superior temporal visual fields) cross. Consequently, bitemporal hemianopsia is the most

common visual field abnormality associated with pituitary adenomas. However, the exact pattern of visual field loss is variable and is affected by the location and flexibility of the chiasm as well as the direction and extent of tumor growth. Large tumors may grow asymmetrically and invade the cavernous sinus or surround an optic nerve, leading to other patterns of visual field changes or loss of visual acuity. It is essential for all patients with pituitary tumors to undergo high-resolution radiologic imaging to evaluate the size and location of the tumor. Formal visual field testing by an ophthalmologist is required to detect subtle visual field changes and should be performed in all patients with suprasellar extension. Longstanding visual field changes may not be reversed by surgical decompression, but dramatic improvements can occur if visual loss is recent.

The normal pituitary is often compressed into a thin rim of tissue by large pituitary adenomas. Hypopituitarism probably results more from compression of the hypothalamic-pituitary stalk than from direct replacement or pressure on the normal pituitary. GH deficiency and hypogonadotropic hypogonadism are particularly common. Slightly elevated prolactin levels (generally <100ng per milliliter) occur in cases of stalk compression because of diminished inhibition by dopamine. It is important not to mistake such tumors for prolactinomas, as they will not decrease in size in response to medical therapy with bromocriptine. Preoperative hypopituitarism caused by a large pituitary mass is reversible in up to half of patients after surgical decompression. Diabetes insipidus (vasopressin deficiency) is rarely caused by pituitary tumors and should raise the suspicion of a craniopharyngioma or other disorders likely to cause hypothalamic dysfunction.


Material
&
Methods

Material and Methods

Twenty-two patients diagnosed clinically as having nonfunctioning pituitary adenomas were analyzed. They underwent surgery and fragments of tumor were collected, fixed in 10% Buffered formalin, routinely processed and embedded in paraffin for sectioning and immunocytochemical staining. Each tumor was stained for the following pituitary peptides: Prolactin, Human Growth hormone, Adrenocorticotropic hormone (ACTH), FSH, and TSH.

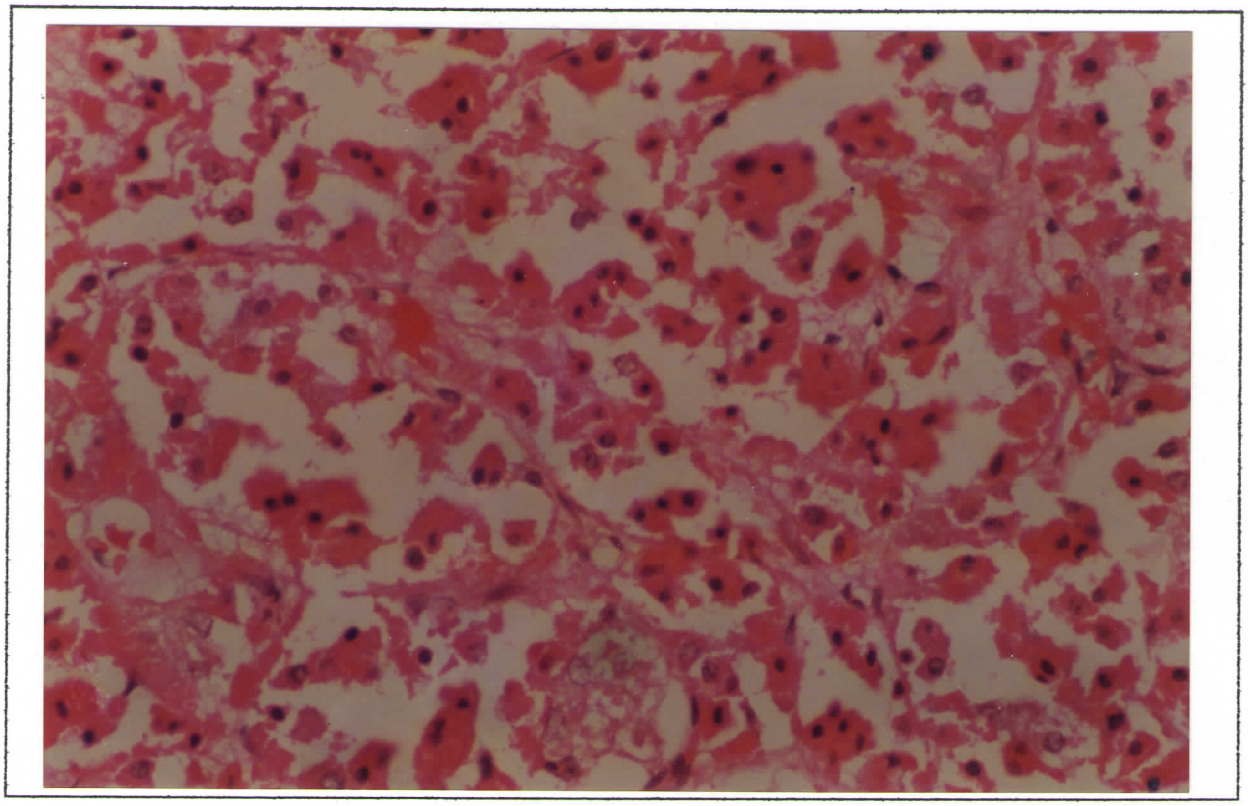
Immunocytochemical staining was carried out on 5 μm paraffin-embedded sections of each tumor by the following method.

Method:

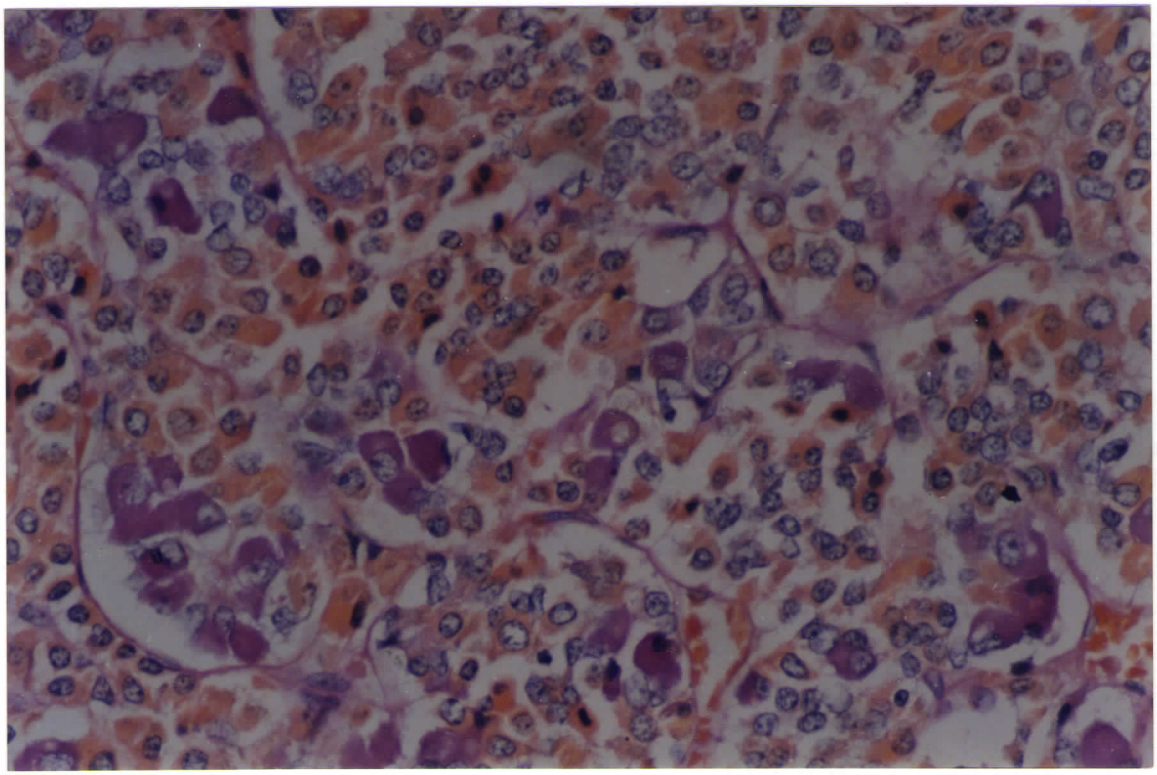
- Preparation of 3-5 micron section in Poly-Lysine coated slides made, deparaffinised and brought to Dist. Water through graded alcohol.
- The endogenous peroxide activity is blocked by treating the slides in 3% hydrogen peroxide and slides are immersed in 0.05 M Tris-buffer PH 7.6.
- Treated with Primary antibody in room temperature in humid chamber for 10-15 min
- Rinsed, washed well in buffer.
- Treated with Biotinlated link antibody (secondary) anti-mouse for 10 min
- Washed well and treated with Strepavidine HRP for 01 min
- Washed with buffer and treated with Chromogen- DAB for 5-min.
- Washed and counterstained the nucleus with Hematoxylin.
- Wash and blue in 0.05% ammonia water.
- Dehydrated, cleared and mounted.

The extent of immunoreactivity was recorded for each hormone on a scale from "-" (no staining) to "++++" (heavy staining).

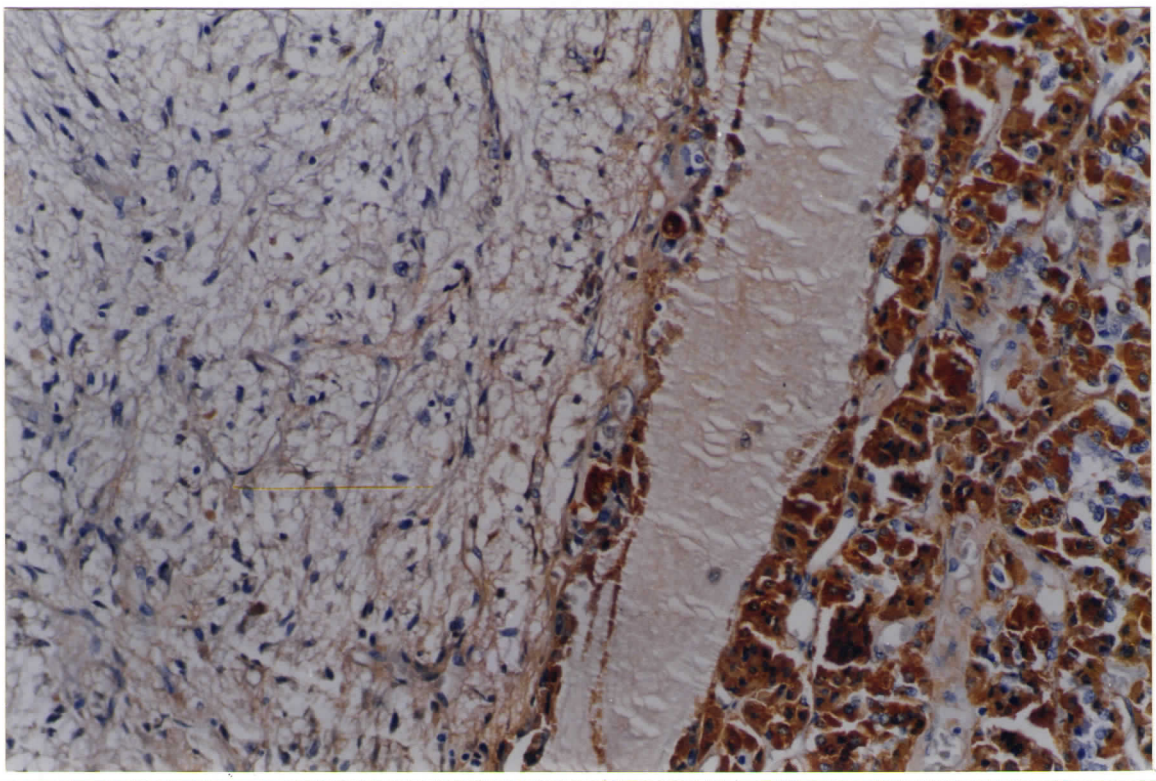
Source: All reagents were available in kit forms from Dako-LSAB@ systems



Photograph 1: Normal pituitary gland; H&E staining



Photograph 2: Normal pituitary PAS Orange staining showing acidophils (orange colour), basophils (magenta) and chromophobes (not stained)



Photograph 3: Normal pituitary Adeno-Neurophyseal junction, showing immunostaining for GH in adenophysis


Observations

Of the twenty-two cases there were 14 men aged between 24 to 55 years (mean 40.36 years) and 8 women aged between 30 to 68 years (mean 46.63 years) with clinically nonfunctioning pituitary adenomas.

Figure 1: Sex distribution

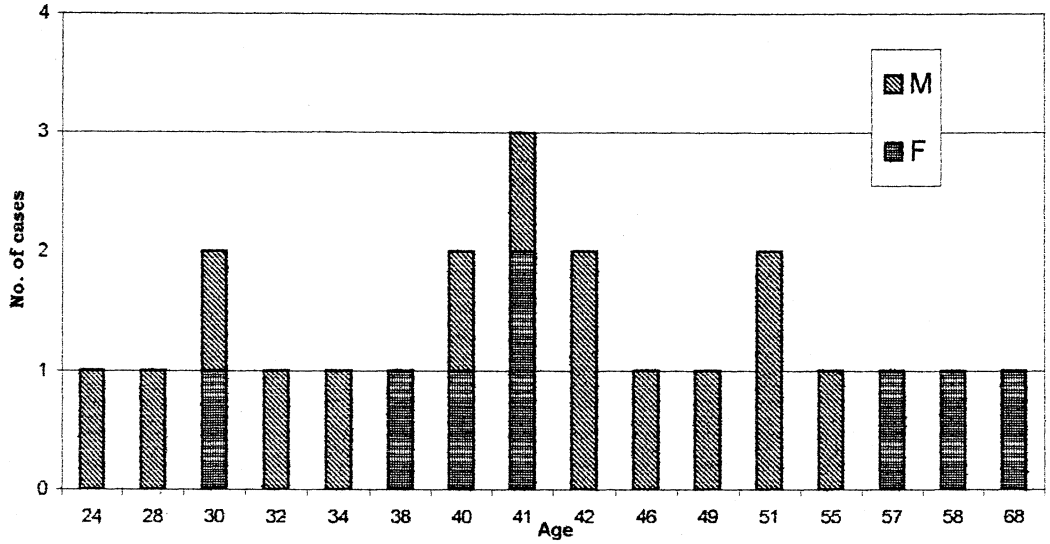
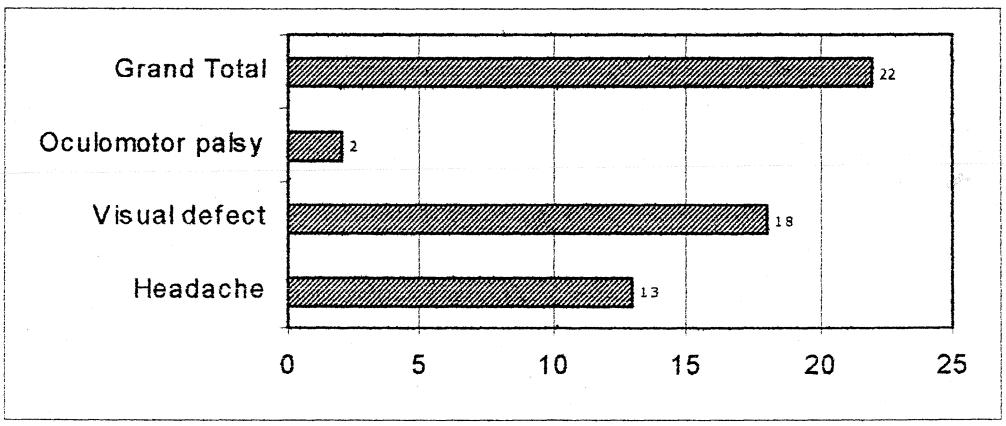


Table 1: Presenting complaints

Sex	Headache	Visual defect	Oculomotor palsy	Total
F	5 (62.50%)	5 (62.50%)	1 (12.50%)	8
M	8 (57.14%)	13 (92.86%)	1 (7.14%)	14
Total	13 (59.09%)	18 (81.82%)	2 (9.09%)	22

Loss of vision was the major presenting symptom – 82%; affecting 93% of the men and 63% of women. Headache was the next common complaint (59%), followed by oculomotor deficit 9%.

Figure 2: Presenting complaint



The preoperative endocrine data is shown in Table below.

Table 2: Preoperative Serum Hormonal levels

Case No	Age (yrs)	Sex	Prolactin (ng/ml)	GH (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	Cortisol	T3 (µg/dl)	T4	TSH (µIU/ml)
1	41	F	38	0.08	9	2.3	6.9	75	5.5	1.72
2	46	M	13.96	0.05			2.5	125	4.35	1.38
3	30	F	63.7	1.4	6.4	1.8	7.1	109.62	4.4	2.83
4	32	M	4.8	0.18	5.4	2.3	25.2	185.92	4.5	0.79
5	58	F	9.3	1.4	75	25	12.9	90	10.1	1.67
6	24	M	11.1	0.5	3	1	2.1	124.62	6.5	3.06
7	42	M	12.6	0.1	43.3	3.9	49.1	131.35	10.3	0.5
8	40	F	2	1	2	2	16	207	11	0.8
9	55	M	16.3	0.7			5.5	72.56	4.6	1.38
10	49	M	10	1	8.4	4.1	5.8	132	8.1	2.4
11	38	F	44.4	0.05	3.65	3.26	10.61	1.32	12.25	1.82
12	68	F	48	0.4	48.6	12.4	4.4	120	6.8	1.2
13	34	M	9.05	0.88	2	2	1	98.2	4.5	2.87
14	42	M	12.1	4	2.4	1.8	6.4	92	6.9	1.4
15	30	M	21.5	1.8	18.2	1.1	4.4	153	8	0.95
16	28	M	8.2	1	1.8	1	4	118	9.8	0.2
17	51	M	24.5	1.1	2.6	1.5	3.1	176	6.8	0.01
18	41	F	31.65	1.63	3.4	1	73	171	7.53	0.97
19	40	M	10.7	2	3.6	2	3.2	2.3	1.3	0.2
20	41	M	11.3	2.6	11	3	4	80	7.1	0.3
21	57	F	6.5	2.8	60.4	18.6	3.8	110	8.8	1.3
22	51	M	18.4	1.3	4.8	1.4	5	86	10.8	0.3
Normal Ranges			3 - 25	0 - 7	Upto 20	Upto 15		86 - 186	4.5 - 12.5	0.4 - 4

Of the 22 cases of nonfunctional pituitary adenoma studied by immunohistochemistry, 9 cases (40.9%) showed immunopositivity to pituitary hormones. One case (case no.11) showed multihormonal immunopositivity- to GH, Prolactin and FSH. Immunostaining features of all these cases are shown in the table below.

Immunoreactive hormones found in 22 patients with Nonfunctioning adenoma

Table 3: Immunohistochemical staining

Case No.	Age	Sex	IHC GH	IHC Prolactin	IHC ACTH	IHC FSH	IHC TSH
1	41	F	-	-	-	-	-
2	46	M	-	-	-	-	-
3	30	F	-	-	-	-	-
4	32	M	-	-	+++	-	-
5	58	F	-	+	-	-	-
6	24	M	-	-	-	-	-
7	42	M	-	-	-	++	-
8	40	F	-	-	-	-	-
9	55	M	-	-	-	-	-
10	49	M	-	-	-	-	-
11	38	F	+++	++	-	++	-
12	68	F	-	-	-	-	-
13	34	M	-	-	-	-	-
14	42	M	+	-	-	-	-
15	30	M	-	-	-	+	-
16	28	M	-	-	-	-	-
17	51	M	-	-	-	-	-
18	41	F	-	-	-	-	-
19	40	M	-	-	-	+	-
20	41	M	-	-	-	+	-
21	57	F	-	-	-	-	-
22	51	M	+	-	-	-	-

Of the 9 cases, which stained positive on immunohistochemistry, 5 cases had FSH immunopositivity (23% of total 22 cases). Next frequent one was for GH (3 cases, 14%). Prolactin was positive for 2 cases (9%) and ACTH positivity in one case. No cases stained positive for TSH. Only one case (case 11) showed positivity for multiple hormones and is depicted in the chart below.

Figure 3: Immunoreactivity staining

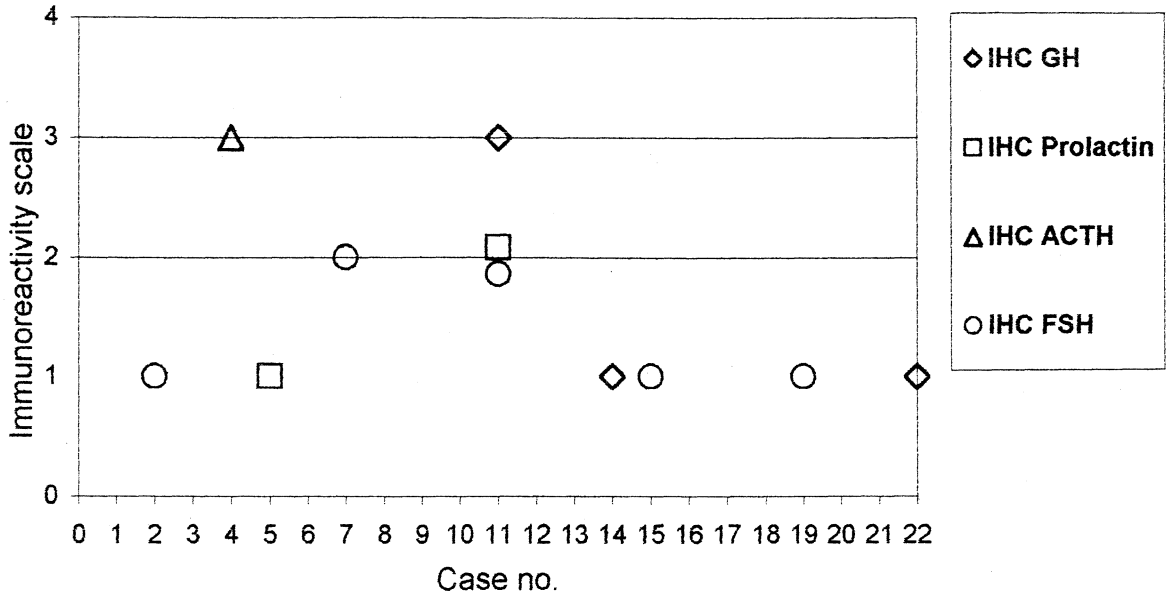


Table 4: Immunostaining positivity table

Immunostaining	Positivity (n=22)	Percentage
FSH	5	22.73
TSH	0	0
Prolactin	2	9.1
GH	3	13.64
ACTH	1	4.55

Following are the charts showing preoperative hormonal values in each case against immunopositivity.

Of the 3 cases, which stained positive for GH, only one case showed marginal increase in serum GH level; but all remained within the normal range.

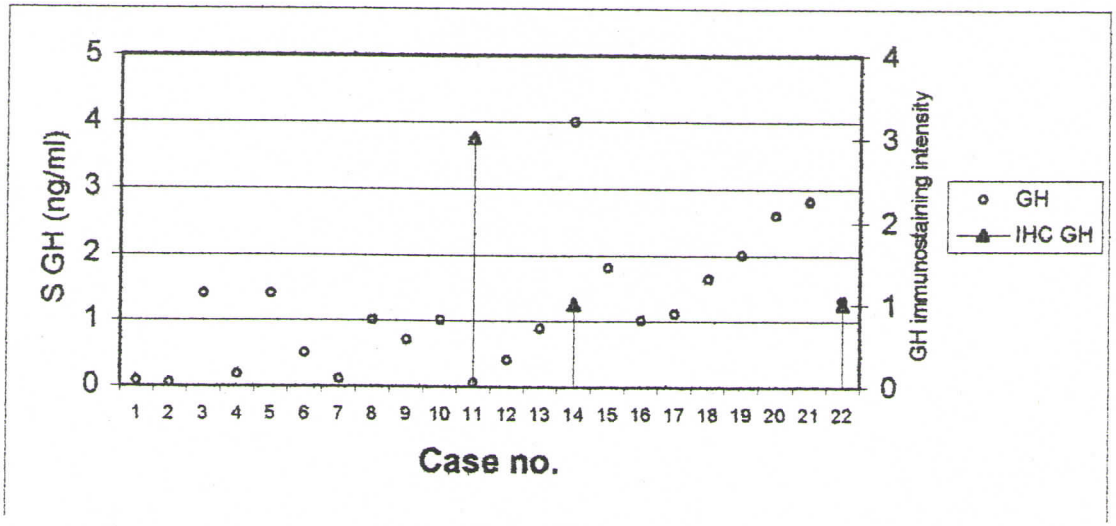
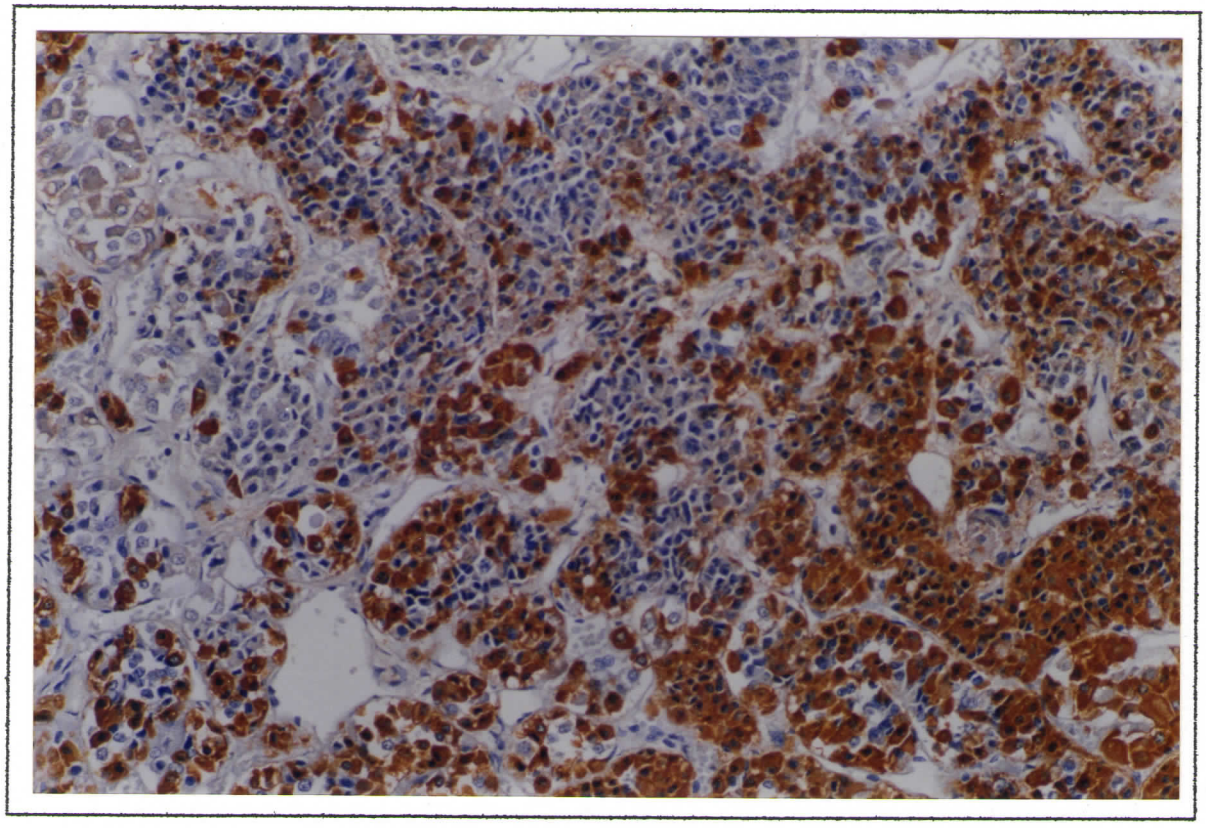
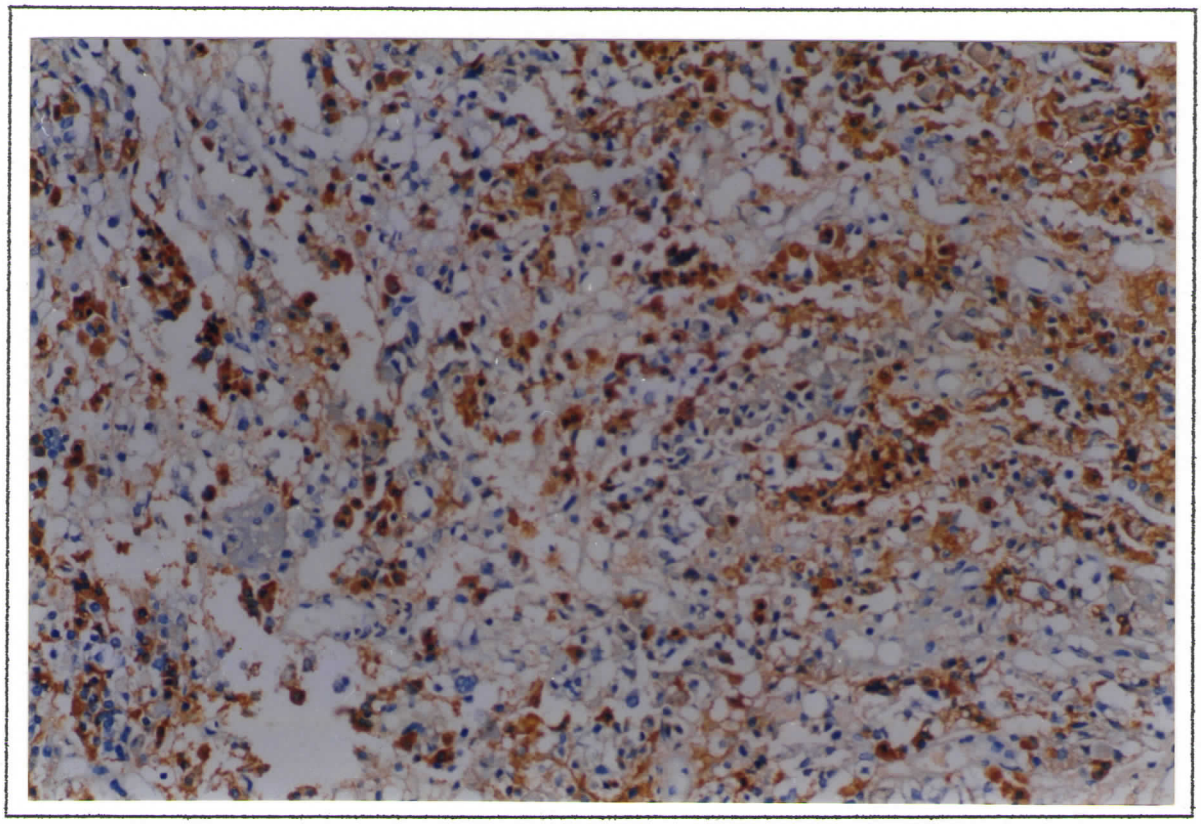


Figure 4: GH values and GH immunopositivity



Photograph 4: Normal pituitary gland showing admixture of GH +ve and –ve cells (Control)



Photograph 5: Pituitary adenoma tumor showing immunopositive GH cells (20X)

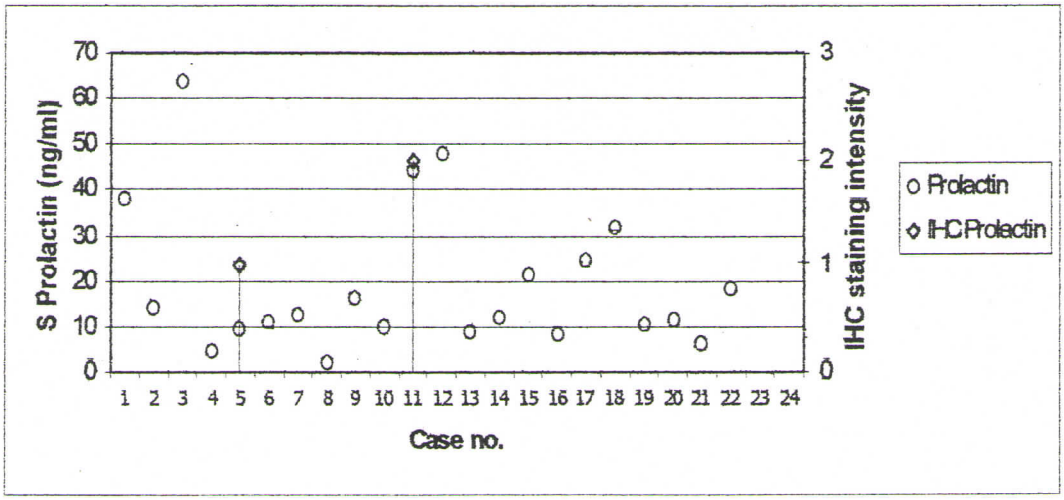
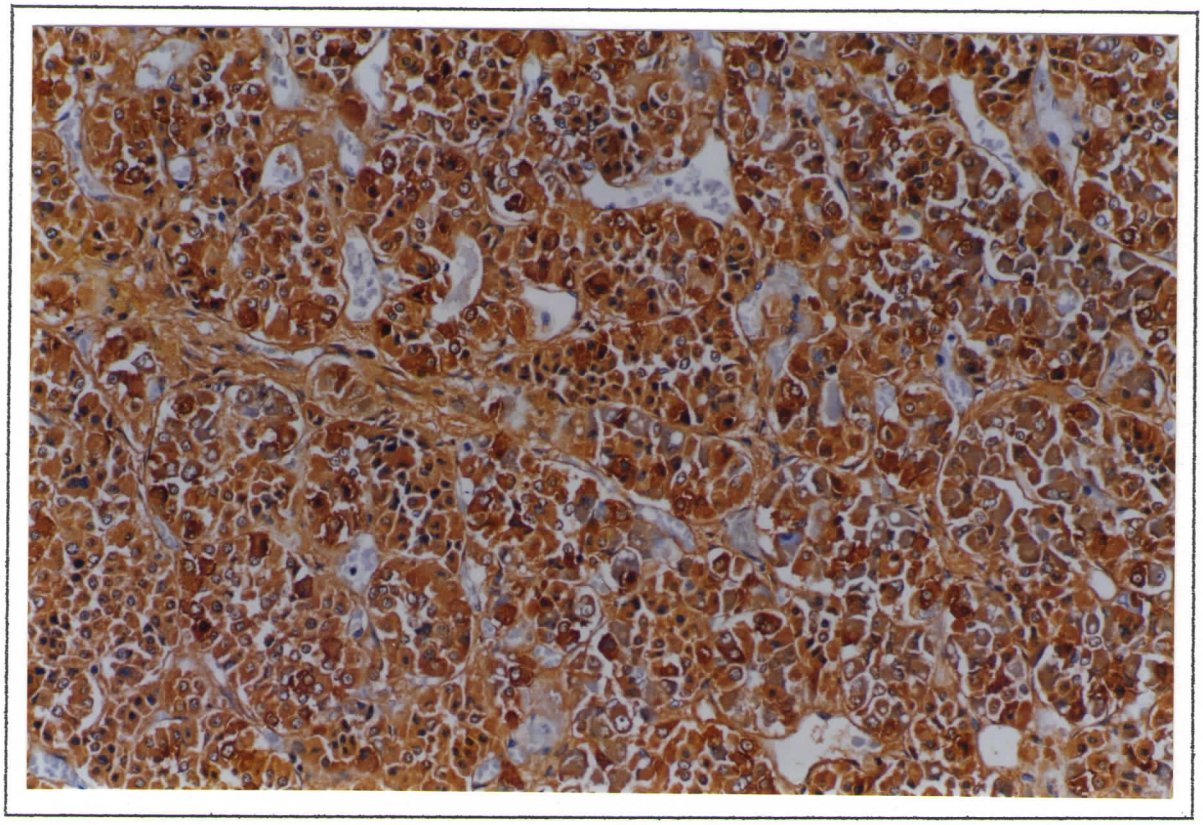
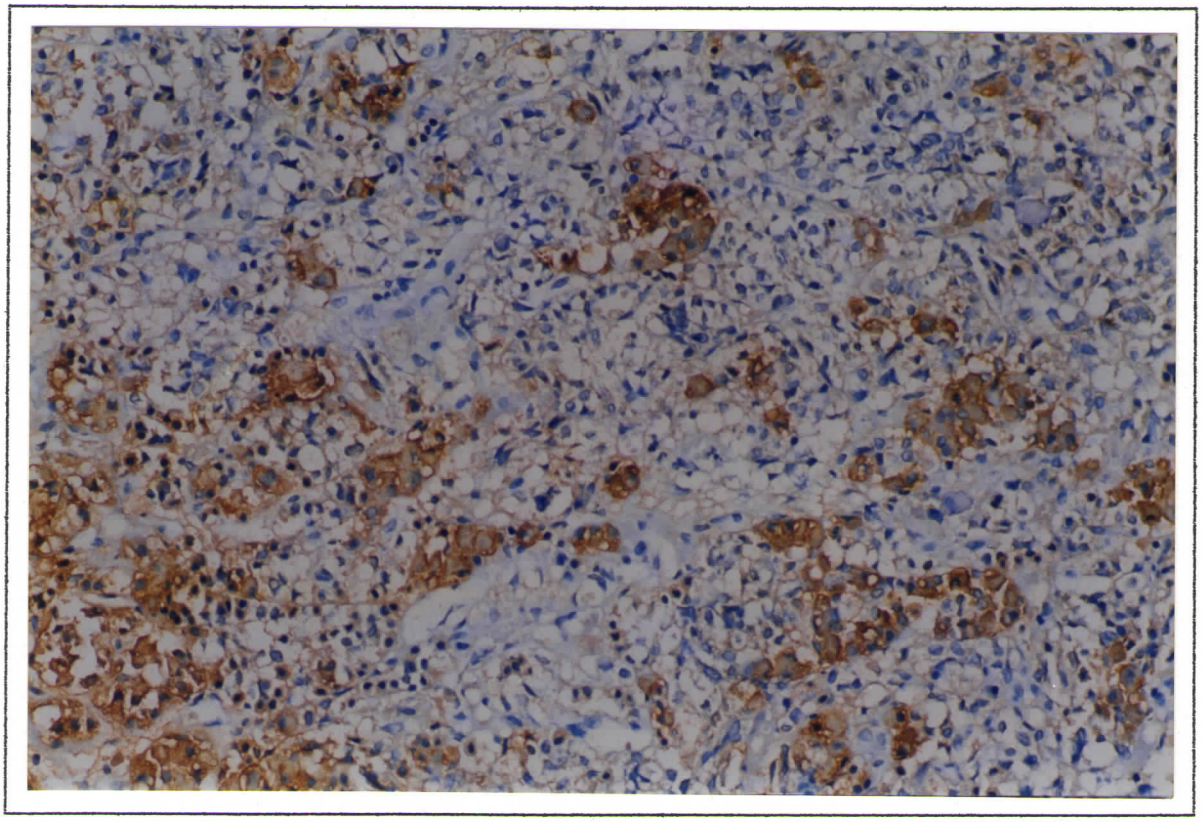


Figure 5: Prolactin values and Immunopositivity



Photograph 6: Normal gland showing positive immunostaining for Prolactin (control)



Photograph 7: Pituitary adenoma showing immunostaining for Prolactin

The five-immunopositive cases for FSH are shown against preoperative serum FSH values. Marginal increase in FSH value is noted in one case (case 7).

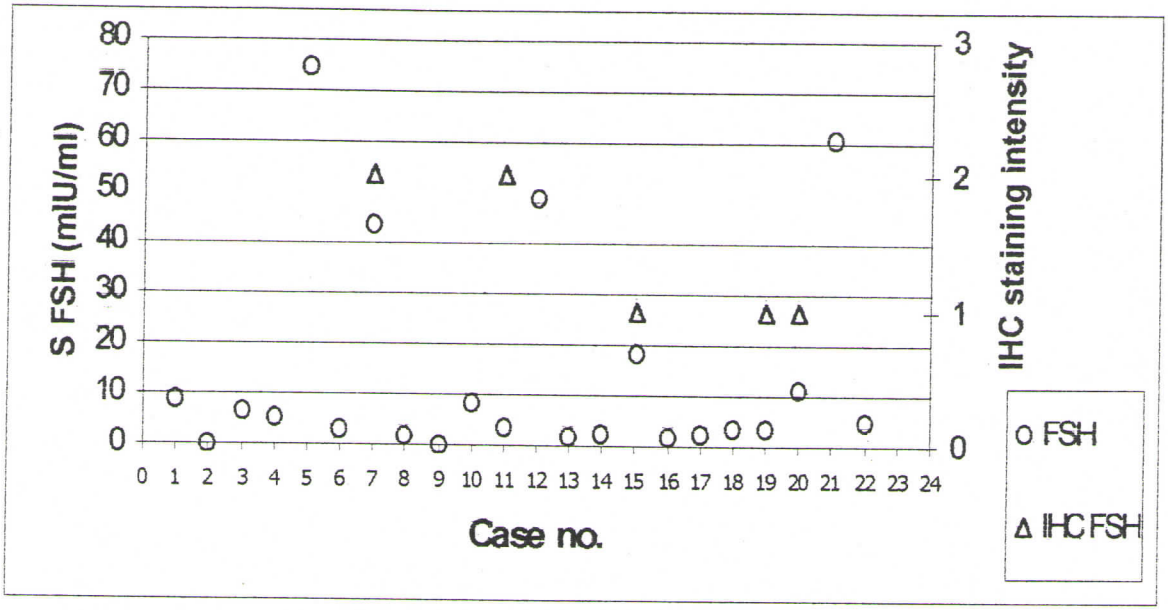
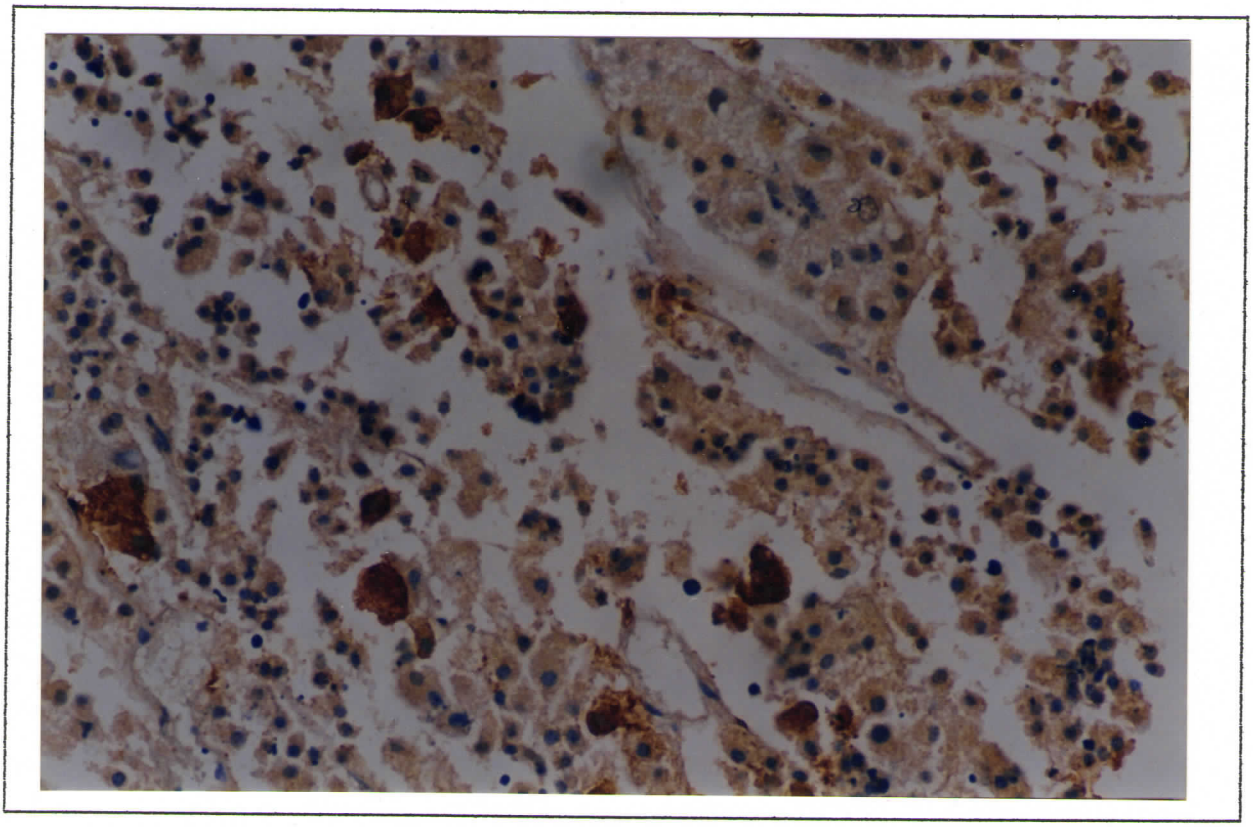
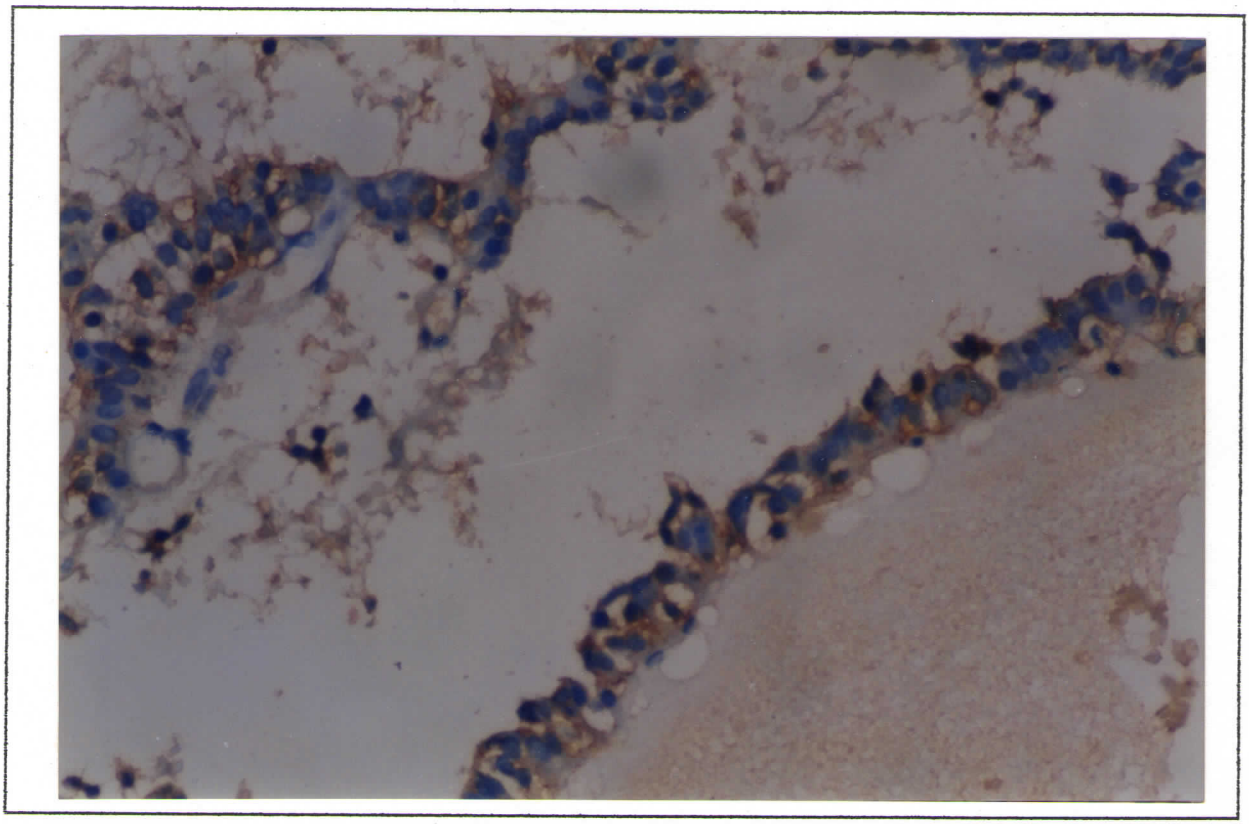


Figure 6: FSH values and FSH immunopositivity



Photograph 8: Normal FSH positive pituitary gland in a female



Photograph 9: Immunostaining for FSH in a pituitary adenoma (40X)

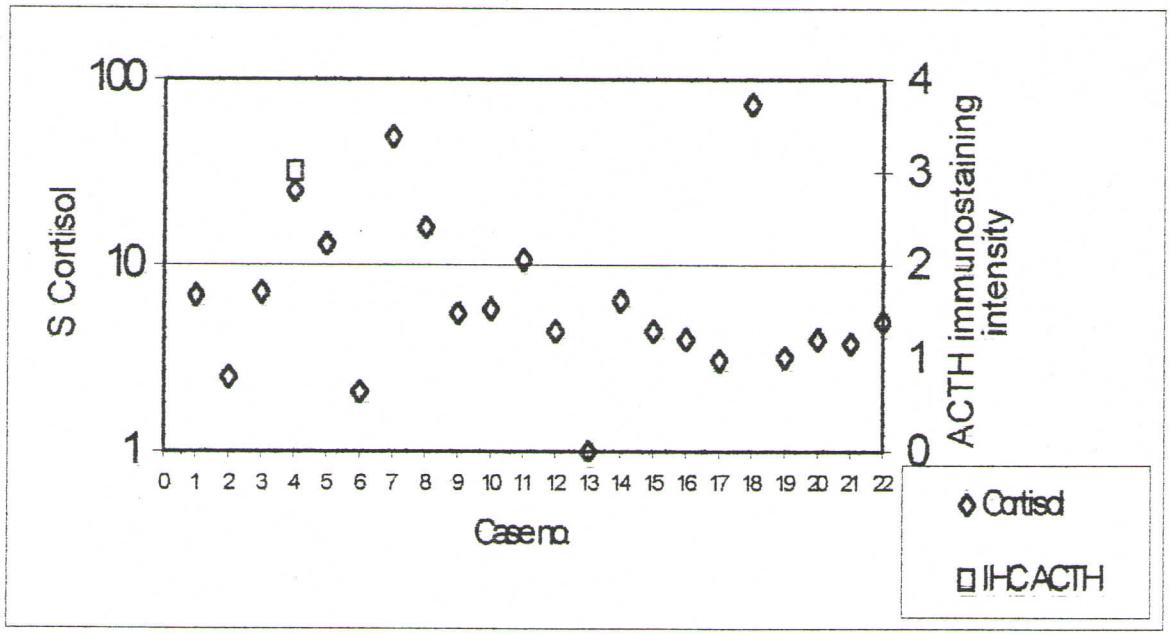
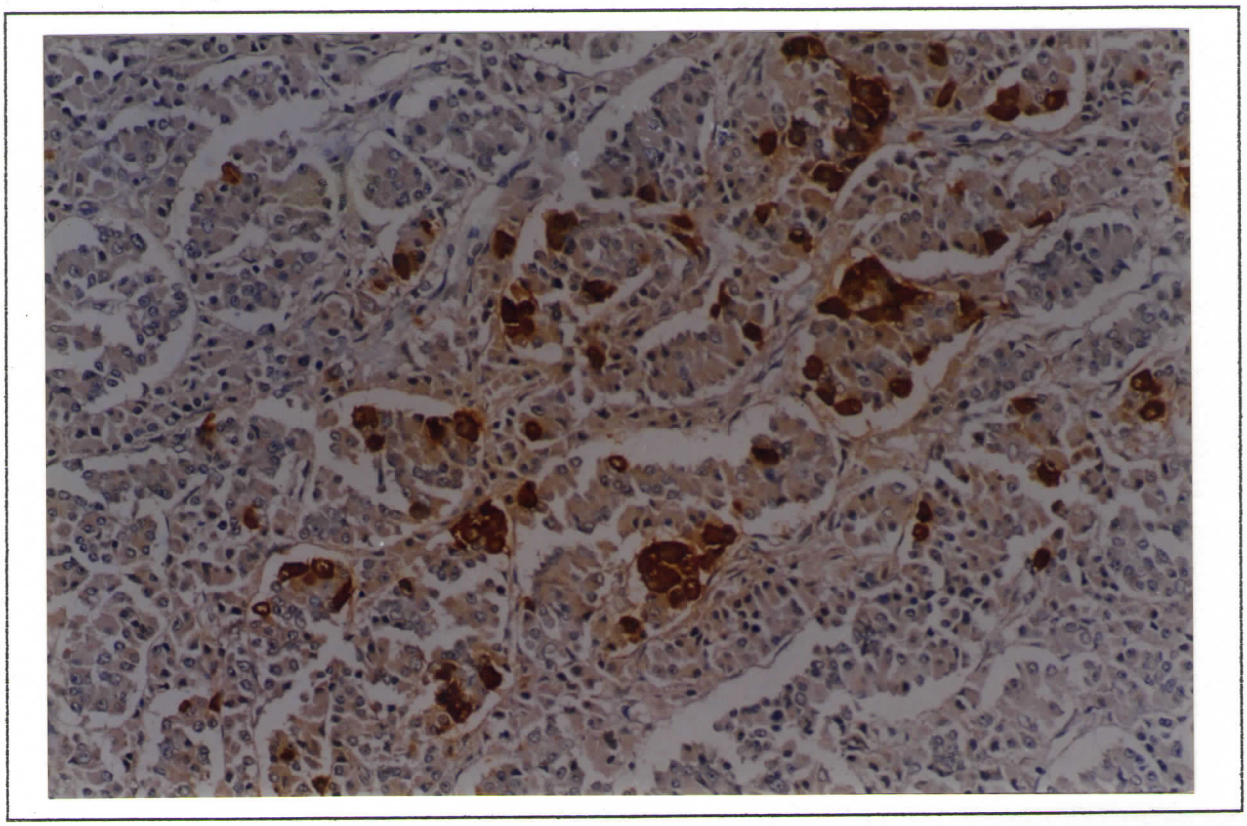
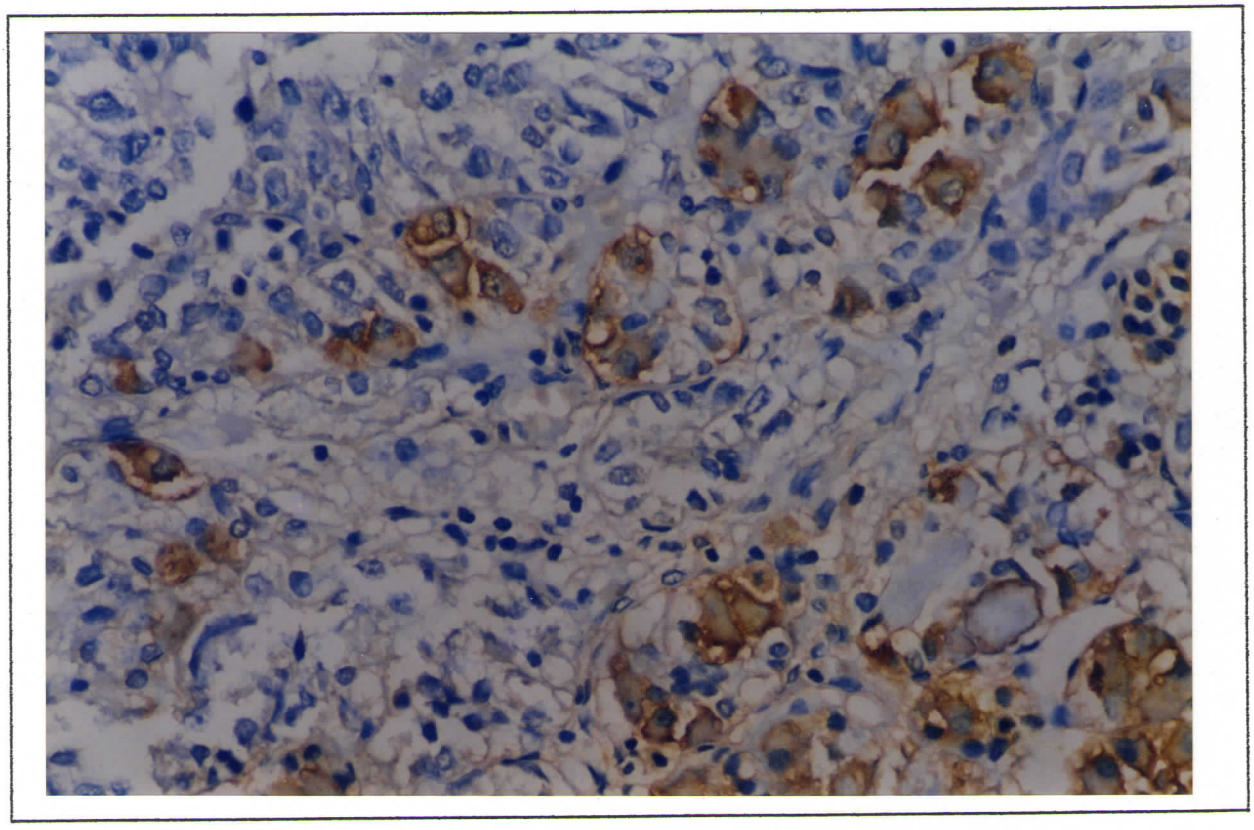


Figure 7: Serum cortisol values and ACTH immunopositivity



Photograph 10: Normal pituitary adenoma showing ACTH immunopositivity (control) 20 X



Photograph 11: Pituitary adenoma showing admixture of ACTH +ve and –ve cells (40 X)

There were no immunopositivity for TSH in any of the cases in this present study. The chart below shows the thyroid profile of the cases studied all of which were within normal ranges.

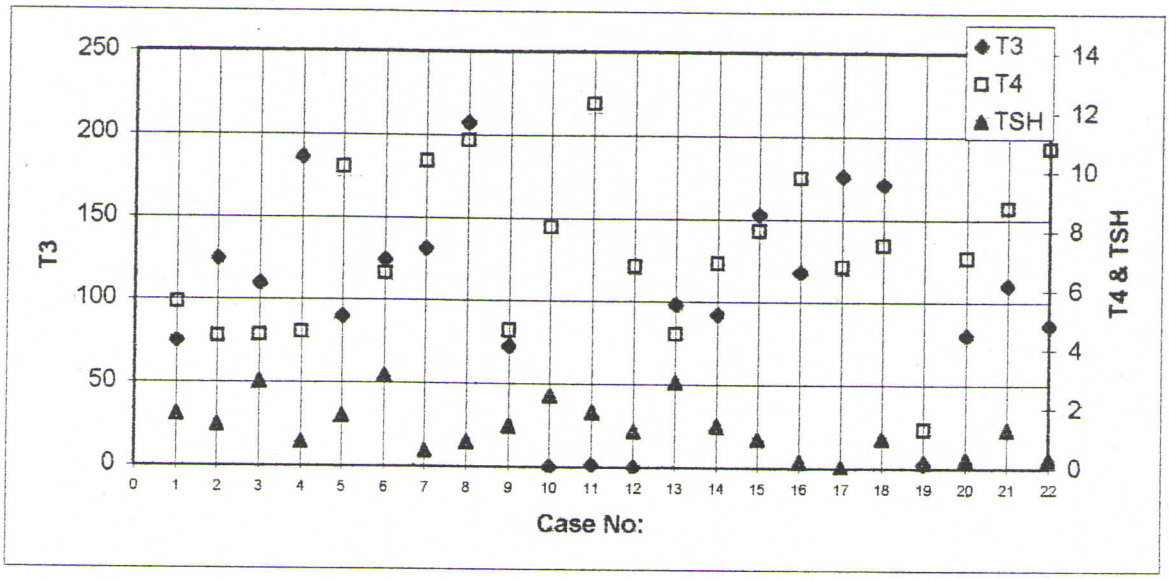
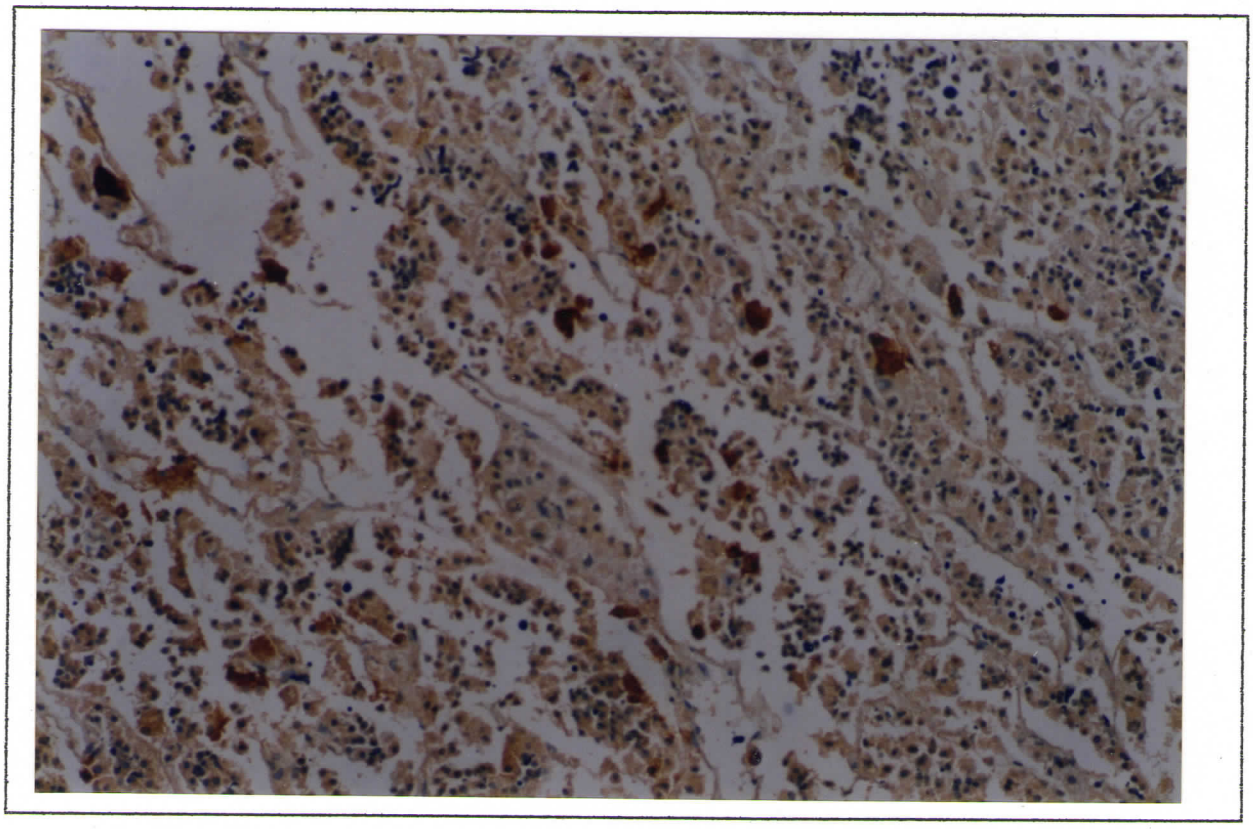


Figure 8: Thyroid function test results; No TSH immunopositivity



Photograph 12: Normal pituitary gland showing TSH positive cells (control) 20 X


Discussion

Discussion

Twenty-two cases of clinically nonfunctioning pituitary adenomas were analyzed in the present study. The male-female sex ratio is 1.8 which is higher when compared to similar other studies where it ranged from 0.8 to 1.4.[2,1,23,10]

The mean age of the patients in this study is 40.4 years for males and 46.6 years for females, which is a decade lower when compared to other studies as is shown in table below. This could explain the lower incidence of immunopositivity for hormones especially the gonadotropins, which occur mostly in patients over 50 years age.

Authors	**Black etal[2]**	**Jameson etal[1]**	**Croue etal[23]**	**Esiri etal[10]**	**Present Study**
No. of patients	37	14	40	48	**22**
Male/Female Ratio	16/21 0.8	7/7 1	22/18 1.22	28/20 1.4	**14/8** **1.8**
Mean age(yrs) Male/Female	54/55.5	48/58	53.3/58.8	49/49	**40.4/46.6**
Visual defect	65%	93%	53%		**82%**
Headache	8%		12.5%		**59%**
Diplopia	4%				**9%**

With respect to clinical presentation, majority presented with visual disturbance (82%) that is comparable to other studies, which ranged from 53% to 93% as is shown in the table above. Headache and/or diplopia was present as an associated complaint in 59 and 9 percentage patients respectively

Immunohistochemical analysis of 22 nonfunctional pituitary adenoma cases in this study showed 41 % immunopositivity to any one or more of the anterior pituitary hormones. This is much lower when compared to other studies which showed more than 60 % positivity except for in one study by Esiri etal[10] where it was42%.

Majority of immunopositive cases in the nonfunctional adenomas in all studies were for glycoprotein hormones. The glycoprotein hormones are heterodimers consisting of two different subunits called alpha and beta. The α-subunit is common to all of the glycoprotein hormones and the unique β-subunits (β-FSH, β-LH, β-TSH) confers biological and immunological specificity to the hormones[24]. Many glycoprotein hormone adenomas secrete uncombined and biologically inert α and/or β-subunits[24]. Interestingly, although FSH is released primarily as intact hormone, LH is secreted largely in the form of uncombined LHβ subunit. This indicates that there are both biosynthetic and secretory defects in glycoprotein hormone producing adenomas and may explain, in part, the rare occurrence of endocrine manifestation by these tumors.

In the present study also, of the 9-immunopositive cases out of the total 22 cases, majority had stained positive for the glycoprotein FSH (5 cases - 22.7%). In all previous similar studies glycoprotein hormones and/or their subunits constituted the majority ranging from 30% to 100%.

We have not done immunohistochemical analysis for LH or for the glycoprotein subunits (α, β-FSH, β-LH, β-TSH). Majority of clinically nonfunctional tumors are presumably of gonadotrophic origin and LH and FSH are often secreted as bioinactive free α and/or β-subunits. Hyper secretion of α-subunit has been well established as an uncommon but consistent phenomenon occurring in 1% to 10% of adenomas.[2,25,26,27] Synder et al[28,29] have shown that some gonadotroph adenomas predominantly produce β-LH subunits. This could explain the low immunopositive cases in this study compared to other studies.

Authors	Black etal[2]	Asa etal[30]	Jameson etal[1]	Croue etal[23]	Esiri etal[10]	Present Study
No. of patients	37	12	14	40	48	22
Immuno positivity	73 %		93 %	62.5 %	41.7 %	41 %
FSHβ, LHβ, α	66.7 %	100 %	78.6 %	37.5 %	20.8 % (FSHβ, LHβ)	22.7 % (FSH)
TSHβ, α	33.3 %	25 %	28.6 %	2.5 %	2.1 % (TSHβ)	0 (TSH)
Prolactin	16.7 %	0	7.1 %	0	14.6 %	9.1 %
GH	2.8 %	0	7.1 %	0	2.1 %	13.6 %
ACTH	8.5 %	0	7.1 %	10 %	12.5 %	4.6 %

GH was positive for 3 cases forming about 14% of the cases. This is much higher with respect to other studies. GH immunopositive staining without clinical evidence of acromegaly was demonstrated in one case by Esiri etal[10] and in 3 cases by Klibanski et al[4].

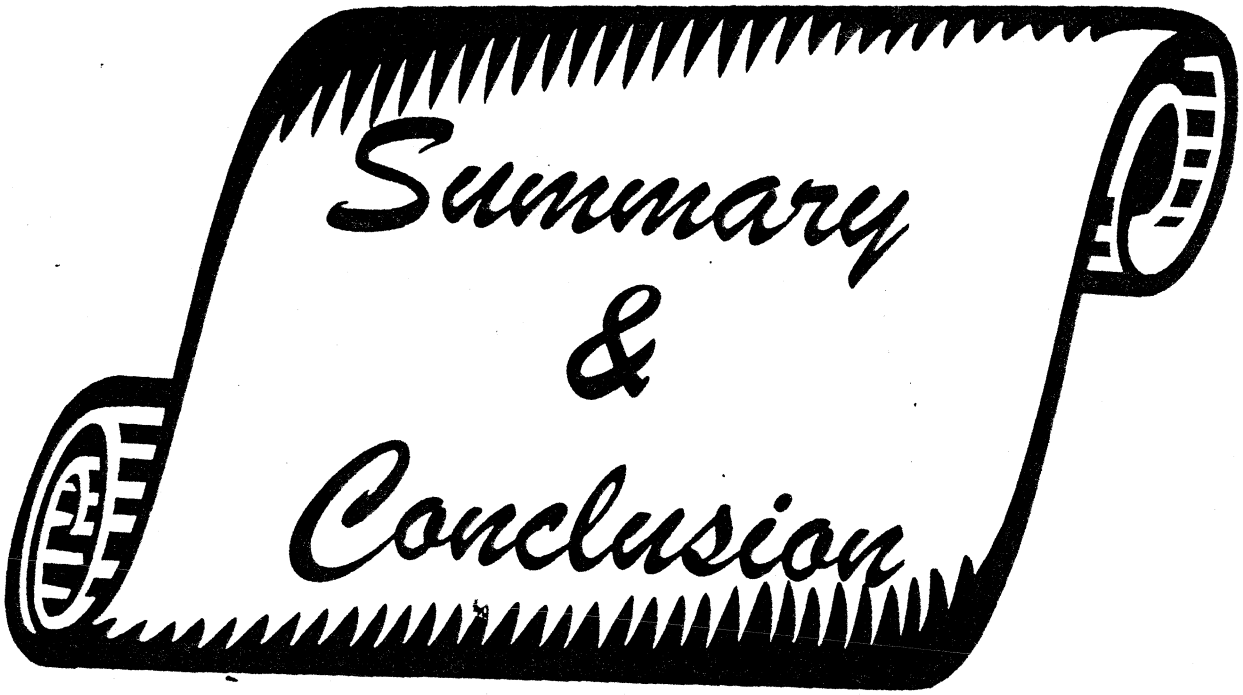
Two cases showed Prolactin positivity (9.1%), which correlates with other studies. Both cases had serum prolactin levels mildly elevated but not beyond 70 ng/ml, suggesting that it is not the result of PRL secretion from the macroadenoma, but from the mass effect of the tumor on either the pituitary stalk of the hypothalamus.

ACTH was positive in one case only (5%). Horvath et al[3], found that 4.3% of tumors staining for ACTH were clinically silent, and small numbers of "silent" ACTH-secreting adenomas have been found in several other series[13,17,18,19].

Immunoreactivity to more than one hormone was noted in only one case - to GH, Prolactin and FSH. In study by Croue etal only one case out of 40 showed

pleurihormonal staining, but the study by Black etal, showed 13 out of 37 cases to be pleurihormonal. Majority were combinations of gonadotropins with thyrotropin or prolactin

However by this present study out of 22 cases, 9 could be further classified by immunohistochemistry to 4 cases of FSH adenomas, 2 GH adenomas, one Prolactin adenoma, one ACTH adenoma and one as hormonal combination adenoma. It is clear that immunohistochemical study using subunit specific assays would help in identifying more tumor phenotypes and in classifying them further.


Summary
&
Conclusion

Summary and Conclusion

Twenty-two cases of nonfunctional pituitary adenomas were analyzed prospectively with respect to clinical presentation and using immunohistochemical techniques. This has helped in assessing the presentation of these adenomas in our patient population and in further characterization of clinically nonfunctional adenomas.

The following conclusions were made from the study.

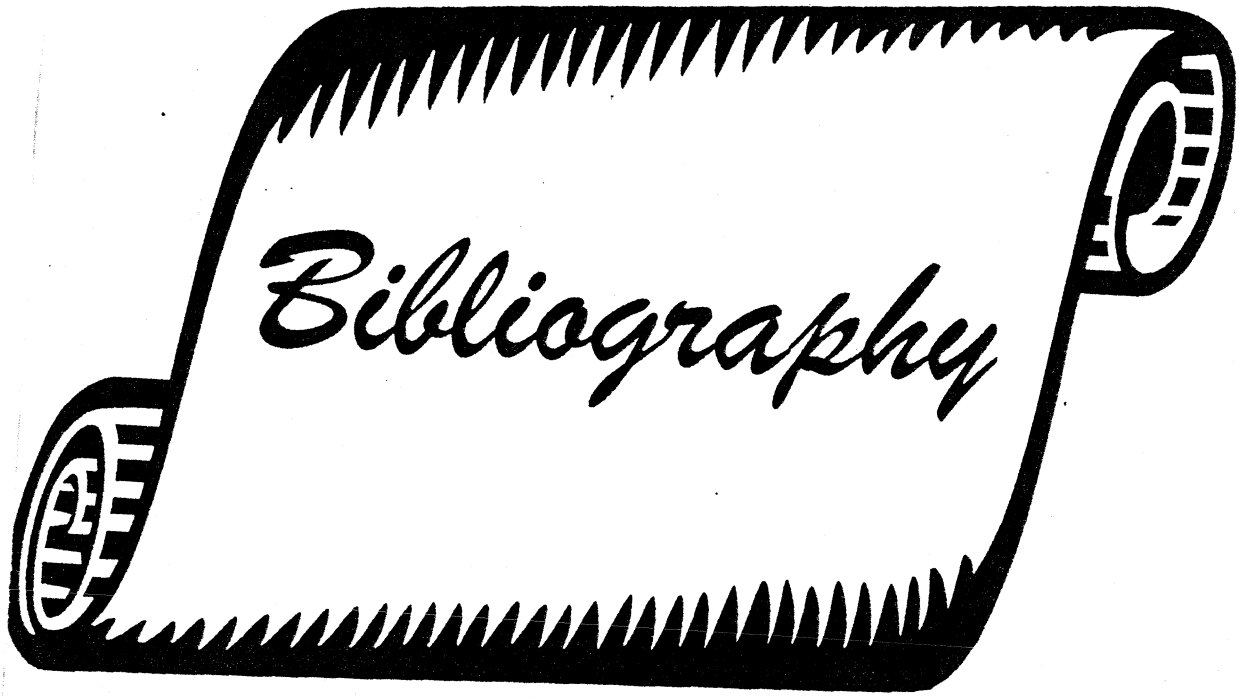
1. As compared to other reported series, nonfunctional pituitary adenomas have been found to occur one decade earlier in our patients.

2. Visual impairment is the commonest mode of presentation (80%) followed by headache and diplopia

3. Gonadotrophic hormone secreting adenomas form the majority of the so called nonfunctional adenomas

4. Immunohistochemical studies can be used to define the cellular phenotype of nonfunctional pituitary adenomas.

5. These analyses may be useful for characterizing the natural history of different subtypes of nonfunctional pituitary adenomas and for selecting patients for newer adjunctive medical therapies.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