ORIGINAL PAPERS / ARTICLES ORIGINAUX

IMMUNOHISTOCHEMICAL ANALYSIS OF PITUITARY ADENOMAS IN A WEST AFRICAN HOSPITAL

ANALYSE IMMUNOHISTOCHIMIQUE DES ADÉNOMES DE L'HYPOPHYSE DANS UN HÔPITAL DE L'AFRIQUE DE L'OUEST

SALAMI Ayodeji [1]
MALOMO Adefolarin O. [2]
SHOKUNBI Temitayo [2]
AKANG Effiong [1]

1. Department of Pathology, College of Medicine, University of Ibadan/University College Hospital, Ibadan, Nigeria
2. Neurological Surgery Division, Department of Surgery, University College Hospital, PMB 5116, Ibadan, Nigeria

E-Mail Contact - SALAMI Ayodeji : ayodejisalami (at) gmail (dot) com

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ABSTRACT

Purpose
Pituitary adenomas are the commonest tumors of the sellar region and constitute 10% to 15% of intracranial neoplasms. The conventional classification of pituitary adenomas is according to the hormone expression of the tumors as determined by immunohistochemical methods. There is paucity of existing research on the frequencies of the various immunohistochemical types in our environment. The purpose of this retrospective study was to determine the relative frequency of specific pituitary adenoma subtypes seen at our hospital over a period of twelve years.

Methods
Forty seven pituitary adenomas received over the study period satisfied the inclusion criteria and their paraffin blocks were retrieved from the archives of the department of Pathology, University College Hospital, Ibadan. Tissue sections were stained with antibodies for Prolactin, Growth hormone, ACTH, TSH, FSH and LH using the streptavidin-biotin-peroxidase method. The tumors were classified using the 2004 WHO classification of pituitary adenomas. Results were tabulated and analyzed using the SPSS statistical software package.

Results
Most adenomas presented between the fourth and seventh decades with a slight female preponderance. Gonadotroph and null cell adenomas were commonest and each constituted 34%, followed by prolactinomas, which accounted for 14.9% of the tumors. There were no thyrotroph adenomas.

Conclusion
The lower incidence of prolactinomas in this study may be due to the use of other therapeutic modes rather than surgical treatment but may also indicate racial differences. However there is a need for further characterization of the null cell adenomas using ultrastructural and molecular studies.

INTRODUCTION

Pituitary adenomas constitute the third most common intracranial neoplasm in adults after gliomas and meningiomas [8,7,1]. Studies from Europe and United States of America have shown a prevalence of 5% to 20% based on cancer registry figures while autopsy and radiological studies have shown a prevalence of 14% and 22.5% respectively[2,9,3, 17,5, 6,24]. Studies done in our environment have indicated a prevalence of 16.8% to 21% [8,7]. Pituitary adenomas are benign tumors but can cause significant morbidity due to pressure effects on neighboring structures, raised intracranial pressure and also due to their functional hormonal effects [13,4,12,15].

The pituitary gland weighs approximately 0.5g but has been called “the conductor of the orchestra” based on the various hormones produced by the anterior part of the gland that controls the activities of many other important endocrine organs in the body [2,17,16,14]. The hormones include prolactin, growth hormone, adrenocorticotropic stimulating hormone, thyroid stimulating hormone, follicle stimulating hormone and luteinizing hormone. Different population of cells produce individual hormones (although rare acidophil stem cells produce Prolactin and growth hormone while many of the gonadotrophs produce both LH and FSH) with each group localized to different parts of the gland [14,22]. Histological classification of pituitary adenomas in developing countries has been based traditionally on tinctorial characteristics of the tumor cells [3,13,18]. According to the 2004 World Health Organization histological classification of endocrine tumors, immunohistochemical assessment of a pituitary adenoma is essential for the optimal management of the patient [13]. Thus pituitary adenomas are now subdivided into six groups based on their immunohistochemical affinities. These include prolactinomas, growth hormone adenomas, corticotrophin adenomas, thyroid hormone stimulating adenomas, gonadotropin adenomas which include FSH and LH adenomas. Prolactinomas are the commonest of the tumors while thyroid stimulating hormone adenomas are rare [5,6].

Pituitary adenomas can either be functional or silent. The functional adenomas often produce symptoms due to the excess hormone produced by the tumor. Silent adenomas are often macroadenomas and although their sub classification is usually identified by immunohistochemical techniques, they do not show clinical effects.

Although many studies have been done on the epidemiology of intracranial tumors in the African setting, a careful search of literature showed no study on the sub classification of pituitary adenomas in our environment.

MATERIALS AND METHODS

This is a retrospective study of all surgical specimens of pituitary adenomas obtained at the University College Hospital, Ibadan over a twelve year period. The record of the age and gender of the patients were obtained from their histology request cards. The archived slides and paraffin wax blocks were retrieved and the slides were reviewed to ascertain the accuracy of the initial diagnoses and to determine if they satisfied the inclusion criteria for the study. The clinical data, gross appearance and microscopic details at the time of initial diagnoses were also reviewed. All cases with histological diagnosis of pituitary adenomas were included in the study. In order to reduce bias and also to identify tumors having cells with multiple antigens each tissue was subjected to all the antibodies as a single batch irrespective of initial H&E assessment. It also served to reveal cases which expressed antigens belonging to more than one histogenetic category. The streptavidin-biotin-peroxidase method was used. Normal pituitary gland was used as positive control for the hormonal stains.

Six serial sections cut at 5 microns each were obtained from the archived paraffin blocks for each case. Antigen unmasking with the heat induced epitope retrieval and the pretreatment methods are shown in table 1.

Data obtained include age distribution of patients with pituitary adenoma, frequency of the different immunohistochemical groups, sex and age distribution of the different immunohistochemical subgroups.

Statistical analysis using Student t-test for comparison of continuous variables and chi-squared test for comparison of discontinuous variables was performed to determine whether there was any association between the various clinical, and immunohistochemical data. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

A total of 47 patients had their samples included in the study. All of the 47 samples had histological diagnosis of pituitary adenoma and also met the study criteria. Thirteen samples were excluded from the study due to inadequate number of sections from the tissue block. Twenty-five (53.2%) of the patients whose samples met the study criteria were females and 22 (46.8%) were males.

The ages of the 47 patients with pituitary adenomas in this study ranged from 9 to 76 years with a mean age of 42 years. The overall peak age of diagnosis of pituitary adenomas occurred in the fifth decade of life (figure 1). The peak age for females was in the fourth decade while that of males was in the sixth decade. However this difference was not significant ($\chi 2 = 9.26$, degrees of freedom (df) = 7, $p = 0.235$).

Null cell and gonadotroph adenomas were the commonest tumors seen with both constituting thirty four per cent of cases each. Prolactinomas were the next most common while corticotroph and somatotroph adenomas were least common (figure 2 and 3). There was no case of thyrotroph adenoma seen in the study. Expression of more than one hormone was common in the gonadotroph adenomas with both LH and FSH positivity in eleven (68.8%) of the sixteen gonadotrophs. In these neoplasms, FSH staining was usually diffuse, whereas LH staining was often focal (figure 4 and 5).

Four gonadotroph adenomas showed exclusive FSH positivity, while one gonadotroph adenoma showed exclusive LH positivity. Growth hormone and prolactin expression was also seen in three cases. One case of corticotroph adenoma also showed focal positivity for TSH.

All the adenomas with exception of gonadotroph adenomas occurred more frequently in females (Table 2). Gonadotroph adenomas occurred three times more frequently in males than in females and the difference was statistically significant ($\chi 2 = 7.74$, degrees of freedom (df) = 1, $p = 0.005$).

Null cell, corticotroph and gonadotroph adenomas occurred more frequently in the third to sixth decades while growth hormone adenomas were seen more frequently in the second and third decades (Table 3). Prolactinomas had a wide age distribution but had a higher frequency in the second to fourth decade.

DISCUSSION

According to the 2004 World Health Organization histological classification of endocrine tumors, immunohistochemical assessment of a pituitary adenoma is essential for the optimal management of the patient [13].

Pituitary adenomas were slightly more common in females than in males in this study. This is in keeping with earlier documentation [20]. Although reasons for this are not entirely clear, it has been postulated that the effect of steroidal hormones that are produced in higher quantity in women may play a role. It is also felt that the earlier appearance of clinical symptoms in women may encourage earlier detection [20].

In the present study, null cell adenomas and gonadotroph adenomas were the two most common pituitary adenoma subtypes identified, each accounting for 34% of the cases. Prolactinomas were the third most frequent pituitary adenoma subtype and accounted for 14.9% of the cases. By contrast to our findings, earlier Caucasian studies have reported a predominance of prolactinomas, which accounted for up to 39% of all cases of pituitary adenoma [11,21,10]. However, the frequency of diagnosis of prolactinoma in the histology laboratory has decreased significantly in developed countries because most patients are managed medically using Dopamine agonists such as bromocriptine and do not require surgery [3,10]. Medical treatment of pituitary adenomas might partly account for the lower frequency of prolactinoma in this study. In more recent Caucasian series, it was reported that gonadotroph adenomas and non-secreting adenomas now account for the majority of tumors seen in histology, which is in agreement with our findings [10]. An additional explanation for the high ratio of null cell and gonadotroph adenomas to prolactinomas in the present study may be the occurrence of genetic variations in pituitary adenomas seen in African as compared to Caucasian patients. On the basis of morphological and ultrastructural features it has been postulated that a significant number of null cell adenomas might actually be gonadotroph adenomas [2,3,17,10]. The combination of the two will account for more than half of the pituitary adenomas diagnosed in the pathology laboratory, as in our study, where both tumors together accounted for over two thirds of cases [2,17,10,23].

Growth hormone adenomas are the least common of the adenomas seen in this study. It occurred predominantly in young adults between the ages of 10 to 30yrs. The gigantism often caused by this tumor may account for its earlier detection in the young. The effect of the tumor is mainly mediated through its effect on the liver with the production of IGF-1[13]. The frequency of this tumor in our study differs from Caucasian series where it is the third most common adenoma after prolactinomas and null cell adenomas. This difference may be due to racial differences although Lloyd et al has noted that the use of medical treatment in somatotroph adenomas accounts for reduced frequency in some series [3,13].

Corticotroph adenomas are the third commonest tumors in this series and occurred more frequently in women. The frequency of ACTH adenomas and sex predilection in this study is in agreement with those in Caucasian series [10].

Gonadotroph adenoma occurred three times more frequently in men than in women in this study. The tumor also occurred in the middle to late decades. Most (68.8%) of the gonadotroph adenomas seen in this study showed immunoreactivity for both FSH and LH. Lloyd et al has documented the higher frequency of gonadotrophs in men as was seen in our study [13]. It has further been postulated that the high levels of gonadotroph hormones produced in women during menopausal period, which often occurs during the peak period of occurrence of gonadotroph adenomas, plays a protective role in women[13,20]. Sixty eight per cent of the gonadotroph adenomas showed dual positivity for both FSH and LH. This was followed by exclusive staining by FSH and one that is exclusively LH. These findings are in keeping with Saeger et al's findings in their study of pituitary adenomas seen in the German registry [2,13,19]. Gonadotroph adenomas which demonstrate positivity for both FSH and LH usually demonstrate stronger and more diffuse staining for FSH, as was observed in this study [13]. Further work however needs to be done to determine the population of cells that stained for the two hormones so as to ascertain whether there is bihormonality of individual tumor cells or whether there are two distinct populations of cells each producing just one of the two hormones in the same tumor.

Most of the null cell adenomas seen in this study occurred from the fourth decade onwards and showed a slight female preponderance. Although our study is in agreement with previous work that has shown very rare occurrence of null cell adenomas below the fourth decade, our series shows a slight female preponderance which is in contrast with Caucasian studies which have shown male preponderance [13]. The explanation for this difference may be a reflection of genetic differences and will require further studies.

No case of thyrotroph adenoma was seen in this series. Thyrotroph adenomas are the least common of the pituitary adenomas and constitute about one percent of all pituitary adenomas [13,24].

Only one case of plurihormonal adenoma was seen in this study. This tumor showed positive staining for both ACTH and TSH which are both from separate developmental pathways. Plurihormonal adenomas have been defined as tumors expressing more than one hormonal phenotype that cannot be explained by normal cytophysiology or developmental mechanisms [13]. Thus tumors expressing LH and FSH or prolactin, growth hormone and TSH are not included in this group. Plurihormonal adenomas are very rare and show no sex predilection [13].

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DISCLOSURES

The authors declare that they have no conflict of interest with regard to this study.

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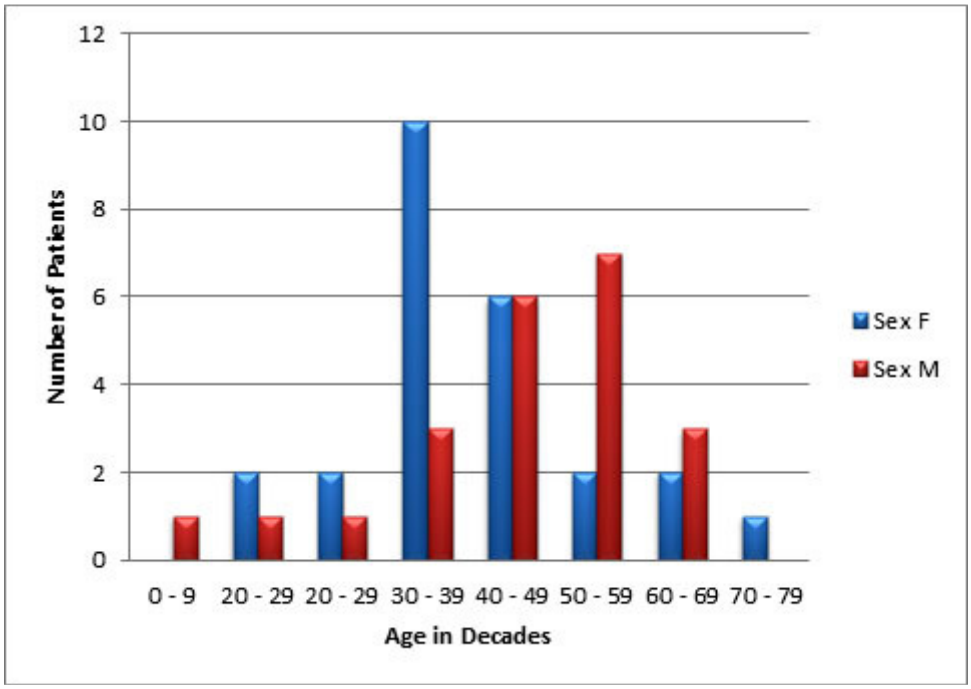

Figure 1
Bar chart showing the age and sex distribution of patients with pituitary adenomas.

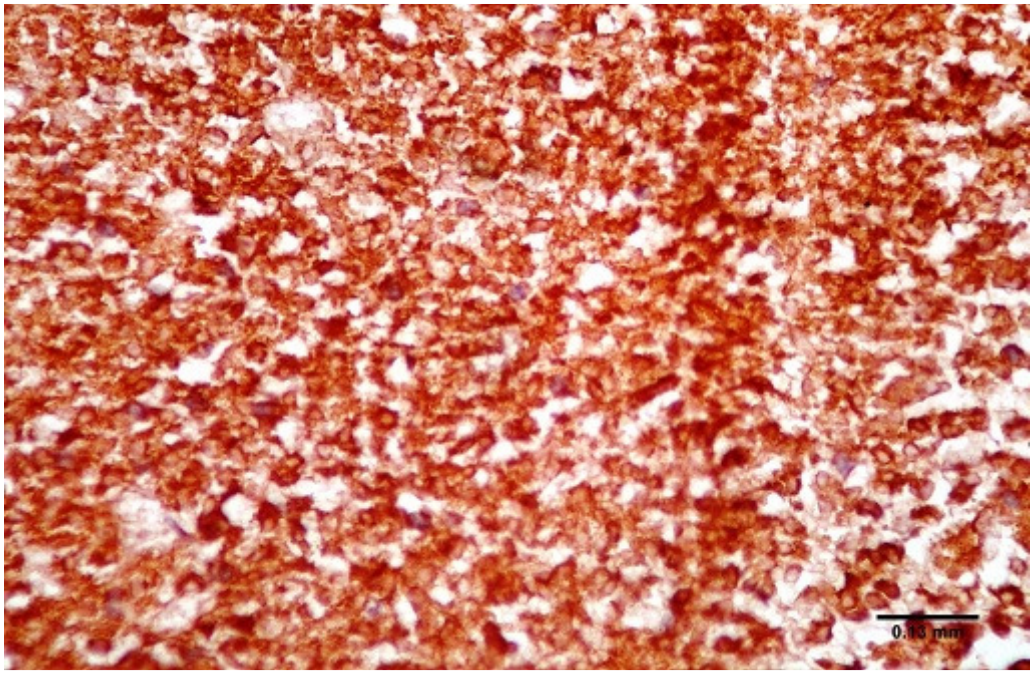

Figure 2
Diffuse staining of Pituitary Corticotroph adenoma with antibody to Adrenocorticotrophic hormone

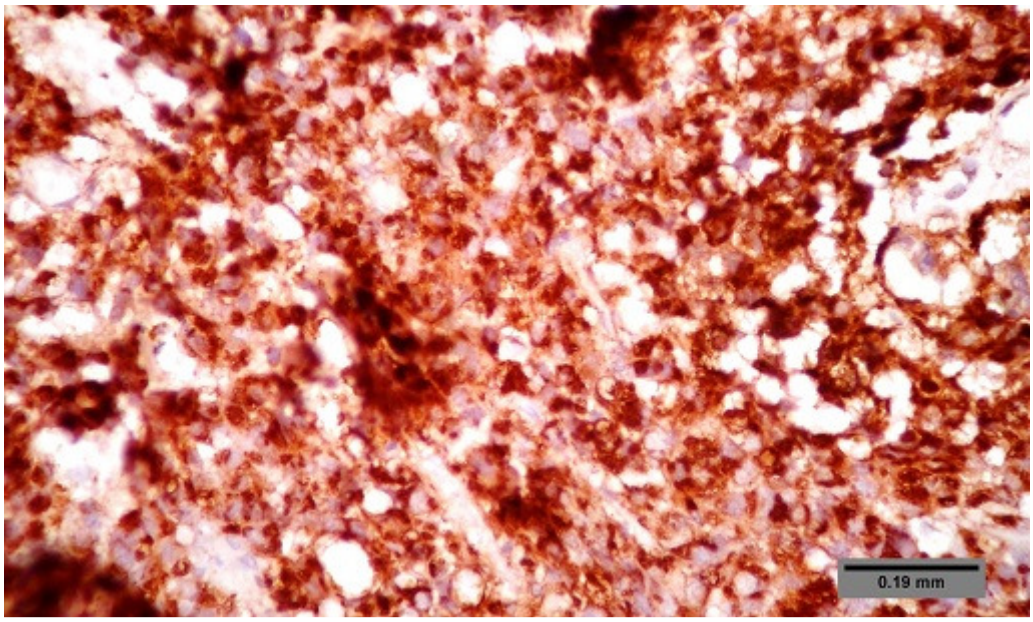

Figure 3
Diffuse staining of prolactinoma with antibody to prolactin

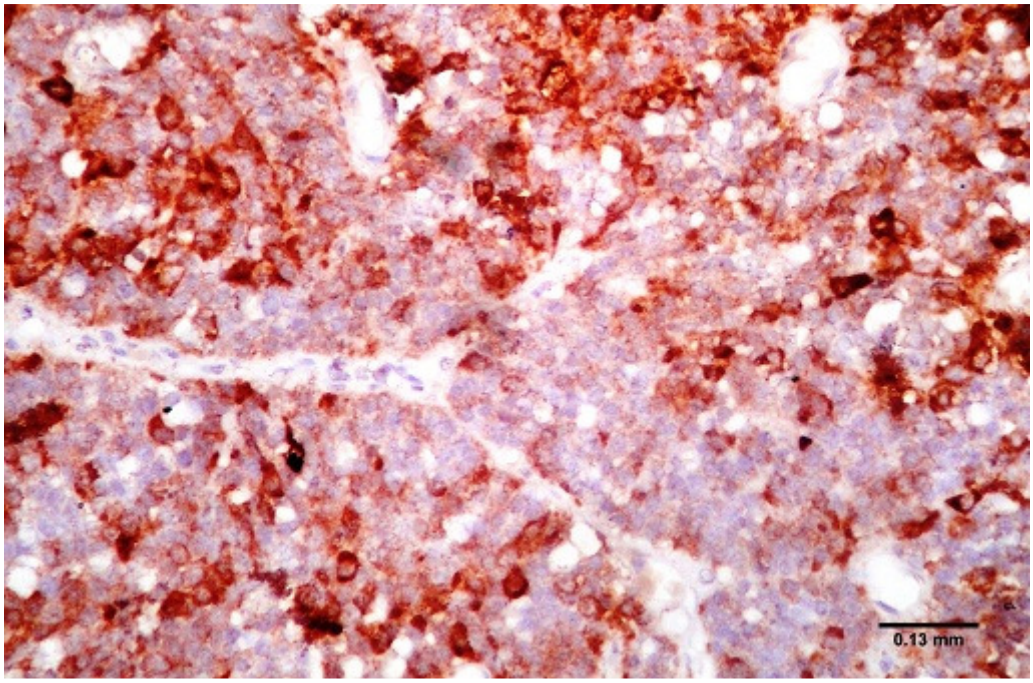

Figure 4
Focal staining of Pituitary Gonadotroph adenoma with antibody to Luteinising hormone

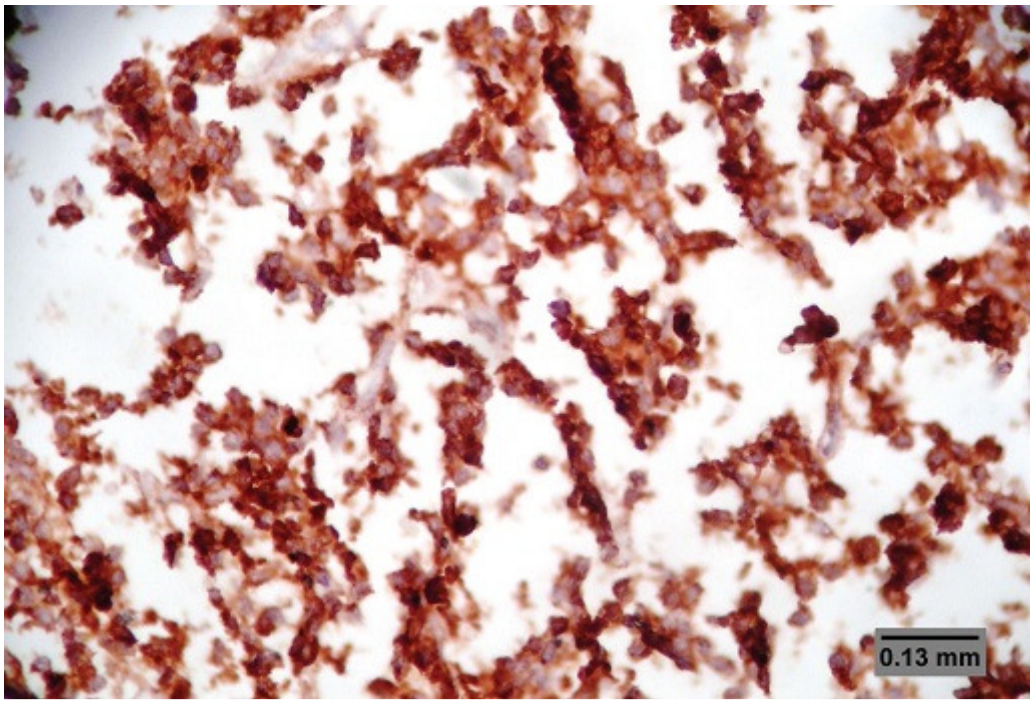

Figure 5
Gonadotroph adenoma positive for Follicle stimulating hormone

Table 1: Immunohistochemical procedure

Antibody	Antibody Dilution	Incubation time	Source	Pretreatment
LH- monoclonal mouse	1:2000	20	ThermoFisher Scientific USA	Boil to 950C
Prolactin- monoclonal mouse	1:800	20	ThermoFisher Scientific USA	Boil to 950C
FSH- monoclonal mouse	1:500	30	ThermoFisher Scientific USA	Boil to 950C
TSH- monoclonal mouse	1:200	20	ThermoFisher Scientific USA	Boil to 950C
ACTH- monoclonal mouse	1:500	30	ThermoFisher Scientific USA	Boil to 950C
GH- monoclonal mouse	1:2000	20	ThermoFisher Scientific USA	Boil to 950C

LH - Luteinizing hormone, FSH - Follicle Stimulating Hormone, TSH - Thyroid Stimulating Hormone, GH - Growth hormone, ACTH - Adrenocorticotrophic hormone

Table 2: Sex distribution of patients with different types of pituitary adenoma

Histological Type	Female	Male	Total
ACTH adenomas	4	1	5
Growth hormone (GH) adenomas	2	1	3
Gonadotrophin (GnH) adenomas	4	12	16
Null cell adenomas	11	5	16
Prolactin (PRL) adenomas	4	3	7
TOTAL	25	22	47

Table 3: Age distribution of patients with different types of pituitary adenoma

Age range (years)	ACTH adenomas	GH adenomas	GnH adenomas	Null cell adenomas	PRL adenomas
0-9	0	0	0	1	0
10-19	0	1	0	0	2
20-29	0	2	0	0	1
30-39	2	0	3	6	2
40-49	2	0	7	3	0
50-59	1	0	3	4	1
60-69	0	0	2	2	1
70-79	0	0	1	0	0
TOTAL	5	3	16	16	7

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