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RELEVANCE OF MOLECULAR MARKERS WITH SPECIAL EMPHASIS ON CLINICALLY NON FUNCTIONING AND BIOLOGICALLY AGGRESSIVE PITUITARY ADENOMAS

Pathology

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ABSTRACT

Background - Pituitary adenomas are the most common tumors of the sellar and suprasellar region. The classification of pituitary adenomas has changed considerably over time. The first attempts at classification relied on hematoxylin and eosin staining of resected tissue. The current 2017 WHO Classification of Pituitary adenomas is defined by hormonal immunohistochemistry of hormones of anterior pituitary and their pituitary specific transcription factors.

Objective – Assess the hormonal profile of pituitary adenomas with emphasis of clinically non-functioning adenomas & analyse the biologically aggressive adenomas on the basis of hormonal profile & proliferation index using IHC markers.

Material & methods –This is a tertiary hospital based study of forty cases of pituitary adenoma. All specimens were fixed in 10 percent formalin and entire biopsy tissue was processed as per standard guidelines. IHC markers were done after H&E microscopy. IHC was done using primary antibody against the these antigens- ACTH, GH, LH, FSH, TSH and MIB1. An integrated diagnosis was done after correlating H&E and IHC results.

Results & Conclusion – Gonadotrophs and Somatotrophs were the most common Pituitary adenomas(25% each) followed by Corticotrophs (22.5%), Lactotrophs(12.5%), Plurihormonal(10%) and Mammosomatotrophs(5%) on IHC subtyping. 25% clinically non functioning and 55% aggressive adenomas were seen on basis of hormonal profile and MIB-1 proliferation index.

KEYWORDS

Pituitary adenoma, Sellar Suprasellar, intracranial neoplasm, Immunohistochemistry Hormonal assessment and MIB-1 proliferation index is important for prognosis and treatment of the patient.

INTRODUCTION

Pituitary adenomas comprises 10-25% of all intracranial neoplasm.[1] They can be clinically functioning & non-functioning.[2][3] Immunohistochemical staining is currently the gold standard for diagnosis and classification of pituitary adenomas as per WHO 2017 classification. Based on Pituitary adenoma's secretory activity they are further classified clinically as[4] (i) Clinically functioning pituitary adenoma (ii) Clinically Non-functioning pituitary adenoma (25-30%). Multiple theories are proposed for the imbalance in clinically non-functioning pituitary adenoma between lack of hormonal hyperexpression and immunohistochemical reactions. Most commonly, caused by low hormonal synthesis or rapid decay before secretion into lysosomes of adenoma cells. Another cause is due to disorder of hormone synthesis or storage and also release of biochemically inactive hormones.[5]

MATERIAL AND METHODS

This is a tertiary hospital based study. A total of forty cases of Pituitary adenomas who got IHC done were included in the study and subtyped according to WHO 2017 classification.

Institutional ethical committee approval was obtained prior to study. Written and informed consent was obtained from participants.

IHC was done using primary antibody against the these antigens- ACTH, GH, LH, FSH, TSH and MIB1.

RESULTS

Total 40 cases of Pituitary adenoma were evaluated for Hormonal profile

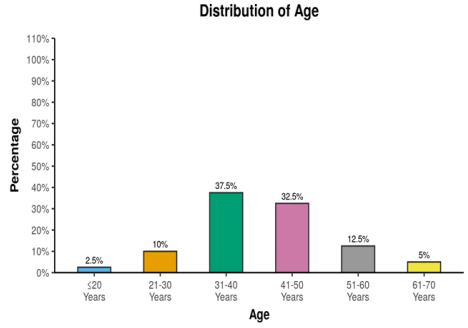

Fig-1- Distribution of Pituitary adenoma cases according to age

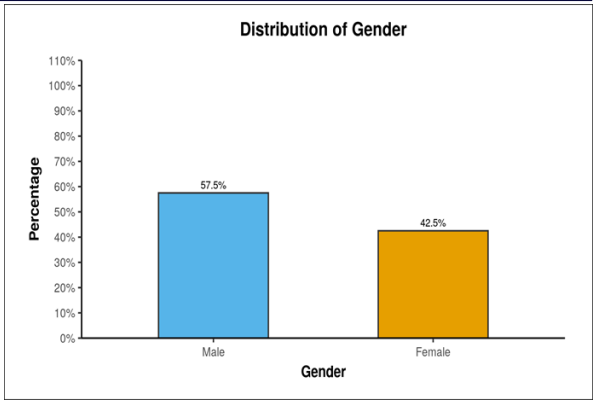

Fig-2- Distribution of Pituitary adenoma cases according to sex

Table 1: Distribution of the study subjects in terms of Clinical Activity (n = 40)

Clinical Activity	Frequency	Percentage
Clinical Functioning	30	75.0%
Clinically Silent	10	25.0%
Total	40	100.0%

75.0% of the participants were Clinical Functioning. 25.0% of the participants were Clinically Silent.

Table 2: Distribution of Clinically functioning study subjects (n = 30)

Clinical Manifestation	Frequency	Percentage
Acromegaly	11	36.66%
Cushing Disease	7	23.33%
Hyperprolactinemia	5	16.66%
Recurrent	4	13.33%
FSH Secreting	2	6.66%
Hyperthyroidism	1	3.33%
Total	30	100.0%

Acromegaly was the most commonly encountered manifestation in clinically functional adenomas seen in 36.66% of study subjects followed by Cushing disease in 23.33% study subjects.

Table 3: Distribution of the Study subjects in Terms of IHC Interpretation (n = 40)

Interpretation	Frequency	Percentage
Somatotroph	10	25.0%
Gonadotroph	10	25.0%
Corticotroph	9	22.5%
Lactotroph	5	12.5%
Plurihormonal	4	10.0%
Mammosomatotroph	2	5.0%
Total	40	100.0%

25.0% of the case subjects were interpreted as Somatotroph. 25.0% of the case subjects were interpreted as Gonadotroph. 22.5% of the case subjects were interpreted as Corticotroph.. 12.5% of the case subjects were interpreted as Lactotroph. 10.0% of the case subjects were interpreted as Plurihormonal. 5.0% of the case subjects were interpreted as Mammosomatotroph.

Table 4: Distribution of the case subjects in terms of Silent Adenomas (n = 10)

Silent Adenomas	Frequency	Percentage
Silent Gonadotroph	5	50.0%
Silent Corticotroph	2	20.0%
Silent Lactotroph	1	10.0%
Silent Plurihormonal	1	10.0%
Silent Somatotroph	1	10.0%
Total	10	100.0%

50.0% of the case subjects were Silent Gonadotroph. 20.0% of the case subjects were Silent Corticotroph. 10.0% of the case subjects were Silent Lactotroph. 10.0% of the case subjects were Silent Plurihormonal. 10.0% of the case subjects were Silent Somatotroph.

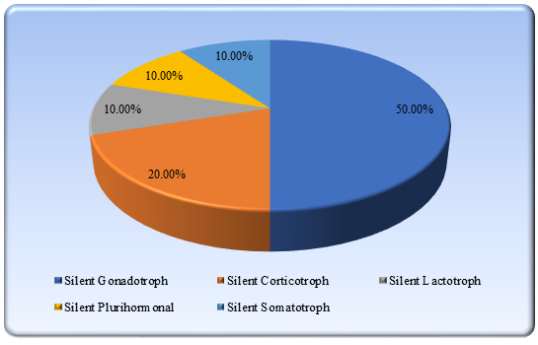

Fig-3- Distribution of the case subjects in terms of Silent Adenomas

Table 5: Association Between MIB-1 and IHC Results (n = 40)

IHC Results	MIB-1 LI		
	≤2 %	>2 %	Total
Somatotroph	7 (26.9%)	3 (21.14%)	10 (25.0%)
Gonadotroph	3 (11.5%)	7 (50.0%)	10 (25.0%)
Corticotroph	8 (30.7%)	1 (7.1%)	9 (22.5%)
Lactotroph	3 (11.5%)	2 (14.2%)	5 (12.5%)
Plurihormonal	4 (15.3%)	0 (0.0%)	4 (10.0%)
Mammosomatotroph	1 (3.8%)	1 (7.1%)	2 (5.0%)
Total	26 (100.0%)	14 (100.0%)	40 (100.0%)

50% of cases subjects with MIB-1 LI >2% were gonadotrophs, 21.14% were somatotrophs, 14.2% were lactotroph and both Corticotroph and mammosomatotroph were 7.1%.

Table 6: Distribution of Biologically aggressive Pituitary adenomas

Interpretation	Frequency	Percentage
Lactotroph in Male	4	28.5%
Recurrent adenomas	4	28.5%
Plurihormonal adenoma	4	28.5%
Silent Corticotroph	2	14.3%
Total	14	100.0%

Two of the lactotrophs in males and all recurrent pituitary adenomas were biologically aggressive as well as had MIB-1 LI > 2.

Table 7: Association Between Aggressive adenomas (biologically aggressive & high proliferaive index) and IHC Results (n=22)

Interpretation	Frequency	Percentage
Gonadotrophs	7	31.81%
Lactotrophs	4	18.18%
Plurihormonal adenoma	4	18.18%
Corticotroph	3	13.63%
Somatotroph	3	13.63%
Mammosomatotroph	1	4.54%
Total	22	100.0%

Seven out of twenty two aggressive adenoma were gonadotrophs including three of them being recurrent with high MIB-1 LI and other four had MIB-1 LI >2

DISCUSSION

Pituitary adenoma arises from one pituitary cell type or more than one cell type and is the most common tumor of the sellar region. Adenomas lack reticulin supported acinar structure of the gland, growth of tumor compresses the adjacent normal gland and its acinar structure and reticulin microskeleton that results in a surgical pseudocapsule which encases the adenoma separating it from the normal gland.[6] Clinical symptoms of pituitary adenomas depend upon their hormonal activity, size and immunohistological subtype.

Male predominance of 57% was observed in our study of 40 cases, however, the biggest study conducted to our knowledge for gender related occurrence of pituitary adenoma by T Mindermann and C B Wilson concluded that frequency of pituitary adenoma differ greatly in their male to female ratio.[7]

Incidence of cases of pituitary adenoma in premenopausal female were more as compared to postmenopausal female in our study. Similar findings were observed by Hemminki, Försti, and Ji with more incidence of females in reproductive age group.[8][9]

Prevalence of silent adenomas in our study was 25% of the total cases. Due to large number of undiagnosed cases of silent adenomas there is a large variation in past studies done. The most recent study of Juliana Drummond et al in 2019 gave a range of 22-54% of incidences of silent adenomas.[10]

Only one case of Silent adenoma was below 40 years of age in our study, indicating an increase in incidence of silent adenomas with age. Georgia Ntali and John A.Wass did an extensive literature review and published a retrospective study in 2017. Their result yielded the peak occurrence of clinically non-functioning adenomas from fourth to eighth decade, thus supporting our study.[11]

Total ten somatotrophs (25%) were diagnosed in our study. Somatotroph adenoma are adenomas which show immunoreactivity with GH. They are often hormonally active and leading to GH excess and tend to occur in older age groups. Acromegaly is associated with 95% of cases GH producing adenoma.[] GH adenomas arises from PIT-1 lineage cells. GH levels tend to fluctuate among individuals, so serum IGF1 levels are done which are stable.

Ten Gonadotrophs were seen our study (25%). Gonadotroph adenomas produce either FSH, LH or both immunohistochemically. They are believed to arise spontaneously. Most gonadotrophs produce symptoms related to mass effects caused by tumor. Among premenopausal women menstrual disorders can be seen. Gonadotrophs may present with impotency in man.

Silent Gonadotrophs are most common in our study accounting 50% of all non-functioning adenoma. Our study was in concordance with study by Arvind Rishi, Mehar C Sharma et al in which they observed almost 64% of silent gonadotrophs of all clinically non-functioning adenomas.

Nine cases (22.5%) in our study were Corticotrophs. Corticotroph adenoma expresses ACTH on immunohistochemistry. They arise from TPIT-1 lineage hypophyseal cells.[11] Most corticotrophs are biochemically and clinically functioning, producing Cushing syndrome. Cushing syndrome was first described by Harvey Cushing in 1912.[85]. Silent Corticotrophs are also considered as an aggressive form of pituitary adenoma.

Two corticotrophs out of nine were clinically non-functioning, accounting upto 22.22% of total cases of corticotroph.

Five Lactotrophs (12.5%) were seen in our study, though they are the most common type of pituitary adenoma with 30-35% of cases. But their incidence in surgical series is low due to higher rate of success in pharmacotherapy. Lactotroph adenomas show immunohistochemical positivity with PRL. Lactotroph adenomas are frequent cause of ovulatory disorders among women and erectile dysfunction in men. Their pathogenesis is relatively unknown. Although experiments show role of estrogen in development of lactotroph adenoma.

Four Plurihormonal adenomas (10%) were seen in our study. Plurihormonal adenomas are rare forms of pituitary adenoma with unusual immunohistochemical combination which cannot be explained by cytodifferentiation. Plurihormonal adenomas can present with acromegaly, hyperprolactinemia, hyperthyroidism or cushing disease. They can also be clinically silent.

One silent plurihormonal adenoma seen in our study is expected to be PIT-1 positive silent adenoma as they are generally silent. All Plurihormonal adenomas were advised PIT-1 testing as, PIT-1 positive plurihormonal adenomas are considered as biologically aggressive adenomas independent of MIB-LI as the criteria for aggressiveness.

Close follow up was advised to the patient of plurihormonal adenomas as previous study by Erikson D et al of Plurihormonal adenomas showed a recurrent and invasive disease in a median follow up of 50 months.

Out of total of twenty two aggressive adenomas (55%), fourteen cases were aggressive only on the basis of increased MIB-1 LI and fourteen cases were diagnosed as aggressive on the basis of immuno histochemistry as per WHO 2017 criteria. They were in the category of biologically aggressive adenomas. Of these biologically aggressive adenomas six cases also had increased MIB-1 LI- Two lactotrophs in males and four recurrent adenomas.

Certain pituitary adenomas are considered as biologically aggressive adenoma irrespective of MIB-1 LI on basis of gender, hormonal activity, histological and immunohistochemical findings such as Lactotrophs in males, Sparsely granulated somatotroph, silent corticotroph, Pit-1positive plurihormonal adenoma and recurrent adenoma.

Four recurrent pituitary adenomas (25%) were seen in our study. MIB-1 LI was found to be high in all of the four recurrent adenomas. Study by Manash Bora, Radhey Shyam Mittal et al yielded similar results.

In our study, MIB-1 LI was >2% in 40% of clinically non functioning adenomas with one silent lactotroph having MIB-1 LI of 12-15%. Wolfsberger et al, considered MIB-1 LI as gold standard for relevance in post-operative management of clinically non-functioning adenoma. Ekramullah et al found that there was an inverse correlation of Ki-67 with tumor doubling time in regrowing non functioning adenoma.

CONCLUSION

Pituitary adenoma are frequently encountered tumours, silent pituitary adenomas represent a challenging group of tumors.

Our study signifies the importance of immunohistochemistry markers for diagnosing clinically silent adenomas and biologically aggressive adenomas. An integrated evaluation of tumor biopsy by light microscopy and immunohistochemistry is needed to diagnose and classify pituitary adenomas as per latest 2017 WHO classification. From clinical point of view, it is necessary to know the hormonal profile as certain types of pituitary adenoma are designed as aggressive adenomas in respect to their gender, size and hormonal activity such as Prolactinoma in males, silent corticotrophs, PIT-1 positive plurihormonal adenoma and recurrent adenomas.

MIB-1 LI is long considered as gold standard for assessing the cell proliferation and biological aggressiveness of the tumor. MIB-1 LI of >2 % is of prognostic significance.

Correlation of immunohistochemistry and proliferative index with clinical history may be important in further adjuvant therapies which may be needed in certain pituitary adenomas. Postoperative Radiotherapy is needed in tumors with high proliferative index.

We recommend a multidisciplinary approach which include Surgeon, Physician and Pathologist is necessary for diagnosis and optimal management of pituitary adenoma patients.

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