

Evaluation of the frequency of gonadotroph adenoma with immunohistochemistry among surgical specimens of pituitary adenoma in Isfahan, Iran

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Abstract

Introduction: Gonadotroph adenomas are a type of pituitary adenomas. It is now clear that these adenomas are relatively more frequent than previously thought.

Objectives: The aim of the present study is to investigate the prevalence of gonadotropin pituitary adenomas.

Patients and Methods: Specimens of surgically resected of 188 patients with pituitary adenomas were collected. In this study chromogranin A was used for immunostaining and identifying gonadotroph adenomas.

Results: Immunostaining turned out to be positive in 93 specimens out of 188. Data analysis indicated a correlation between gender and gonadotropin adenomas prevalence ($P = 0.041$), since of 93 positive specimens, 57.6% were males.

Conclusion: Gonadotroph cells are commonly seen in pituitary adenoma patients. Men are at higher risk of developing this type of tumor.

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Introduction

The pituitary gland is a small size neuroendocrine gland, surrounded with the bony midline depression of sphenoid bone, sella turcica. Hypophysis consists of two characteristically different lobes; anterior lobe (adenohypophysis) and posterior lobe (neurohypophysis) (1). The pituitary gland has the control of, most other endocrine glands' function, through hormone production. Hypophysis function, itself is regulated by hormones of the hypothalamus (stimulating and inhibitory hormones) and also hormones originating from its target glands (2).

Adenohypophysis is composed of five distinct types of cells. These cells are lactotrophs, somatotrophs, corticotrophs, thyrotrophin and gonadotrophs which produce LH and FSH. Each cell named above produces a specific trophic hormone.

Pituitary adenomas comprising 15% of all intracranial tumors, and are benign tumors originating from the adenohypophysis (3-6).

There are several systems of classifications for pituitary adenomas. In one classification

Key point

In a study on specimens of surgically resected of 188 patients with pituitary adenomas, we found that gonadotropin immunohistochemistry was positive in 49% of pituitary adenoma cases and the prevalence is higher in male subjects.

system, pituitary adenomas are classified as functional and nonfunctional (hormonally silent) but according to 2017 WHO classification of pituitary adenomas these adenomas are classified based on their cell lineage; hence they are classified as: somatotroph adenoma, lactotroph adenoma, thyrotroph adenoma, corticotroph adenoma, gonadotroph adenoma, null-cell adenoma and plurihormonal and double adenomas (7).

Gonadotroph adenomas as the name imply originate from gonadotropin cells. Most of the gonadotroph adenomas are non-functional types. The minority of these tumors are the functional ones producing an active form of gonadotropins (LH and FSH) (8, 9). Gonadotroph adenoma's

hormone production usually is not high enough to cause hypersecretory clinical manifestations (8, 10). Therefore, small size gonadotroph pituitary adenomas are mostly diagnosed incidentally. Unless the tumor grows to a size that induces mass effect such as headaches, visual disturbances (11, 12). Additionally, pituitary apoplexy may be present rarely (9). Hypersecretory signs that patients may present with, including infertility, menstrual irregularities, galactorrhea, ovarian hyperstimulation syndrome (9, 12). As for most types of pituitary adenomas, transsphenoidal surgical resection is the treatment of choice for gonadotroph adenomas (13).

Pituitary adenoma types are diagnosed using a technique called immunohistochemistry (IHC). IHC uses labeled antibodies to visually evaluate tissue markers expression. Diagnosis is made based on labeled antibodies determining the presence of specific tissue markers. *Differential diagnosis* of types of pituitary adenomas is based on the immunohistochemical expression of anterior pituitary specific transcription factors and hormones by tumor cells.

Chromogranin A is a protein of grain family and is expressed and released by endocrine cells. It is also present in granules of normal gonadotroph cells of the anterior pituitary. Therefore, it can be used as a marker in immunohistological studies to identify neuroendocrine tumors of gonadotroph type (14,15).

There are limited studies on gonadotroph adenomas' epidemiology. Although gonadotroph adenomas are among the frequent types of pituitary adenomas, functional gonadotroph adenomas are rare while non-functioning gonadotroph adenomas are the common ones(16, 17), because the surgically resected nonfunctioning adenomas consist of a notable proportion of these tumors (16).

Mete et al retrospectively investigated a total of 1055 surgically resected pituitary adenohypophyseal tumors. In their study, steroidogenic factor-1 (SF1) was used for the immunohistochemical diagnosis of gonadotroph tumors. According to this study, gonadotroph adenomas consisted of 42.5% of investigated specimens which seems to be significant (18).

Objectives

Now it is detected that the prevalence of gonadotroph adenomas is higher than previously was thought to be. Considering the nature of the disease and its good prognosis provided by timely diagnosis and treatment, it seems reasonable to conduct researches in this area. Given the lack of well-established research in this area in Iran, we aimed to investigate the prevalence of gonadotropic adenomas.

Patients and Methods

Study design

The current study is a descriptive retrospective cross-sectional study conducted in a tertiary referral health care

center. A total of 209 patients were included in this study. Subjects were patients suffering from pituitary adenoma who were admitted to the hospital and underwent planned surgery between 2012 to 2019. Patients were excluded from the study if their IHC sample was not enough for staining or the paraffin blocks were unavailable or if they were reluctant to share their data. The verbal and written informed consent was obtained from eligible patients. This study was approved by the ethics board of the Isfahan University of Medical Sciences.

Patients diagnosed with pituitary adenoma by endocrinologists were referred to the neurosurgery department for trans-sphenoidal resection surgery. The obtained samples were fixed in formalin 10% and embedded in paraffin. After initial reviews and hematoxylin and eosin staining these paraffin blocks were stored at 4°C in the pathology bank of the health care center. For this study 4µm, thick cuts were obtained from pre-prepared blocks, slices were deparaffinized and rehydrated in a series of xylene and ethanol and then dried. After antigen retrieval was done, due to immunostaining for chromogranin A, specimens were incubated with mouse monoclonal antibodies against human chromogranin A in a dilution of 1:20. These antibodies are isolated from human pheochromocytomas. Human pancreatic tissue specimens served as a positive control.

After preparing the samples the interpretation was performed blindly, without knowledge of the endocrine characteristics of the patients.

Ethnic consideration

The study was in accordance with the Declaration of Helsinki and its later amendments. This paper was the MD thesis of Fatemeh Roustaei, at the Isfahan University of Medical Sciences. This study was approved by the ethics committee affiliated with this university (IR.MUI.MED.REC.1398.723). All the participants were informed about the study aims and procedures.

Statistical analysis

This data was then transferred into SPSS (statistical package for the social science v25.0, SPSS Inc. Chicago, IL). Quantitative data were analyzed using descriptive frequency analysis. To check the relation between the results, age and gender chi-square and Pearson's correlation tests were performed with P value > 0.05.

Results

This retrospective cross-sectional study was performed at Al-Zahra hospital, Isfahan. The subjects of this study were patients suffering from pituitary adenoma who underwent pituitary resection surgery. Of a total of 209 patients, only 188 (89.9%) patients have participated in this study, the other 11 (10.1%) patients were excluded due to exclusion criteria.

Around 92 were patients male and 96 were female with

a mean age of 48.05±9.5 years old. The average age of female participants was 46.62 ±11.44 years and the mean age for male patients was 49.5 ±12.68 years. The Pearson's correlation analysis of data on the relation between age and gonadotroph adenomas shows there is no relation between these two parameters.

As mentioned in Table 1, IHC studies for gonadotroph adenomas have shown to be positive in 93(49.4%) patients' specimen out of 188 specimens, representing 41.7% of females and 57.6% of male patient's pituitary adenomas.

From 92 male specimens, 53(56.9%) of them were positive for chromogranin A while 40 (43%) specimens of 96 female specimens were positive. Analysis of the relationship of results with gender by chi-square method indicates a correlation between gender and prevalence of gonadotropin adenomas with $P=0.029$.

Discussion

Our study aimed to investigate the prevalence of gonadotropin adenomas. Chromogranin A is a glycoprotein produced by a variety of endocrine glands and expressed by gonadotroph cells of pituitary glands. Immunohistostaining of surgically resected pituitary adenomas for chromogranin A antibodies binding sites can differentiate gonadotroph adenomas from other types of pituitary adenomas. In the current study, we used chromogranin A as a marker for the diagnosis of gonadotropin adenomas. The results from data analysis show that immunohistostaining for chromogranin A was positive in 106 specimens out of 188. Patients with gonadotroph adenomas were mostly males.

In a study conducted by Mete et al in Canada between 2001 and 2016 the epidemiology of all adenohypophysis adenomas was studied. In this study transcription factors were used for immunohistochemical localization while hormone immunostains were also used to localize markers such as alpha-subunit of glycoprotein hormones (α SU), beta-thyrotropin (β TSH), beta-folliculotropic (β -follicle-stimulating hormone; β FSH), and beta-luteotropin (β -luteinizing hormone; β LH). Additionally, steroidogenic factor-1 (SF-1) and gonadotropin immunoreactivity were used to identify gonadotroph tumors. Results showed that gonadotroph tumors contained 42.5% of all tumors since 63% of them were in males with the mean age of 58 years. In a Chinese study by Liu and colleague, the clinicopathologic features of pituitary adenomas were investigated retrospectively. In this study, gonadotroph tumors were identified by IHC staining with specific antibodies for follicle-stimulating and luteinizing hormones. They found that 93 (37%) cases had gonadotropin adenomas, while 58.5% were male and the remaining 21.5% were female (19). An explanation for why the result of current study is not completely consistent with the aforementioned studies may be the difference of selected markers for IHC studies. Another study in Canada reported an incidence of gonadotroph adenomas to be 9.4%. They used anti

Table 1. Prevalence of gonadotroph in both gender

	Number	Age	Gonadotroph
Male	92	49.5	53 (57.6%)
Female	96	46.6	40 (41.7%)
Total	188	48.05	93 (49.4%)

h β FSH and anti-h β LH as antibodies for immunostaining. They found 8.3% were females and 10.9% were males, highlighting the incidence is slightly higher (20).

In a study by Batista et al in Brazil, it was found out that 27.3% of non-functional pituitary adenomas are silent gonadotroph. Furthermore, resembling other studies, it was more frequent in men (21, 22). In another study by Zada et al incidence, the clinical characteristics, and implications of atypical pituitary adenomas were investigated. Of 121 patients, 30 of them were recognized to have silent gonadotropin adenomas. One patient out of 30 patients had atypical adenoma. This study did not cover the age-specific and gender-specific incidence of gonadotropin adenomas (23).

Conclusion

Our study showed the prevalence of pituitary gonadotrophs adenomas is significantly higher in men. However, in women, it is diagnosed at younger ages.

Limitations of the study

There were some limitations in the current study for example inaccessibility of patients' medical records. Cases with functional gonadotroph adenomas were not differentiated from functional ones. Additionally, having knowledge about the first impression of the disease and the clinical presentations could provide more satisfying results.

Authors' contribution

AB and AN: study design. AA: patient diagnosis and referral. PRD, MD, PH, SE, MH, AN, AP: Pathology and IHC review. FR: data collection and analysis. AZ, SF: Writing and editing. AB: Supervision and revision of the manuscript.

Conflicts of interest

The authors declare that there were no conflicts of interests. The authors are responsible for the writing and content of this article.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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