EXPERIMENTAL STUDY

Subclinical adenomas in postmortem pituitaries: classification and correlations to clinical data

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Abstract

Objective: The aim of this study was to examine pituitary adenomas in a series of postmortem pituitaries by use of modern technologies of immunostaining, to classify the adenomas according to the current WHO classification and to analyse the possible associations to the available clinical data.

Methods: In this study, pituitaries of 3048 autopsy cases obtained from autopsy series of the years 1991–2004 were examined.

Results: A total of 334 pituitary adenomas were found in 316 pituitaries. One hundred and thirty-two sparsely granulated prolactin cell adenomas (39.5%), 75 null cell adenomas (22.5%) and 31 oncocytomas were diagnosed. Forty-six ACTH cell adenomas (13.8%, 27 densely granulated, 19 sparsely granulated) and one adenoma composed of Crooke's cells were detected. Twenty-two gonadotroph cell adenomas (6.6%), seven GH cell adenomas (four sparsely granulated, three densely granulated), one mixed GH cell–PRL cell adenoma, two TSH cell adenomas, five plurihormonal adenoma type I, four plurihormonal adenoma type II and two α -subunit-only adenomas were seen. Six adenomas remained unclassified because the tissue was not contained in all sections for immunohistochemistry. Seventeen pituitaries included multiple tumours. The overall tumour size ranged from 0.1 to 20 mm in diameter. Among 76 adenomas (22.7%), which had a tumour size of ≥ 3 mm, only three tumours were macroadenomas corresponding to a tumour size of more than 10 mm. The evaluation of the available clinical data showed 99 cases of hypertension, 65 cases of diabetes mellitus, six patients with hyper-thyroidism and four with hypothyroidism. No symptoms of adeno-hypophyseal hormone hypersecretion were reported. The statistical correlations to clinical data were discussed.

Conclusions: Adenomas in postmortem pituitaries differ from those in surgical series in proportion of adenoma types and biological behaviour.

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Introduction

Pituitary adenomas are the most frequently encountered tumours in the pituitary gland and represent about 10% of all surgically resected intracranial neoplasms (1). In postmortem series, the incidence ranges from 3.2 to 27% (2–8). Nowadays, the diagnosis of pituitary adenomas has increased as a result of the advances in neuroimaging technologies. Classification of pituitary adenomas has changed within recent decades. First, the adenomas were classified by functional properties into chromophobic, basophilic and eosinophilic tumour types (7). Later classification of adenomas was based on immunohistochemical hormone content (9–11).

In this study, pituitaries of 3048 autopsy cases were examined to detect the incidence of subclinical adenomas in postmortem pituitaries. The aim of this study was to examine adenomas in a large collective of

postmortem pituitaries by use of modern technologies to classify the adenomas according to the current WHO classification (12) and to analyse the possible correlations to the available clinical data.

Materials and methods

Pituitaries of 3048 autopsy cases obtained from autopsy series of the years 1991–2004 were examined. The tissue was fixed with 10% formalin, sectioned three-dimensionally and totally embedded in paraffin. The prepared tissue samples were cut into 5 μ m-thick sections and stained with haematoxylin/eosin and periodic acid/Schiff (PAS).

Immunohistochemical staining followed using an automated staining machine (DAKO Cytomation Autostainer, Hamburg, Germany) applying the avidin–biotin–peroxidase method. The following

Table 1

Adenoma type	Number of tumours	Included cases with multiple tumours	Percentage
GH cell adenoma, sparsely granulated	4	—	1.2
GH cell adenoma, densely granulated	3	—	0.9
PRL cell adenoma, sparsely granulated	132	12	39.5
Mixed GH cell–PRL cell adenoma	1	—	0.3
Plurihormonal adenoma type I	5	—	1.5
TSHoma	2	1	0.6
ACTH cell adenoma, densely granulated	27	3	8.1
ACTH cell adenoma, sparsely granulated	19	1	5.7
Crooke's cell adenoma	1	—	0.3
Gonadotroph cell adenoma	22	—	6.6
α -Subunit-only-adenoma	2	—	0.6
Plurihormonal adenoma type II	4	—	1.2
Null cell adenoma	75	15	22.5
Oncocytoma	31	3	9.3
Unclassified	6	—	1.8
Total	334	35*	100

*17 cases: 16 with 2 tumours and 1 with 3 tumours.

primary antibodies were used: anti-growth hormone (GH)-monoclonal (BioGenex, San Ramon, USA) diluted 1:200; anti-prolactin (PRL)-monoclonal (Immunotech, Marseille, France) diluted 1:400; anti-adrenocorticotrophic hormone (ACTH)-monoclonal (Novocastra, Newcastle, UK) diluted 1:400; anti-thyroid-stimulating hormone (TSH)-monoclonal (Immunotech) diluted 1:5000; anti- β follicle-stimulating hormone (β FSH)-monoclonal (Immunotech) diluted 1:40 000; anti- β luteinizing hormone (β LH)-monoclonal (Immunotech) diluted 1:5000; anti- α subunit-monoclonal (Immunotech) diluted 1:1500; anti-S-100 protein (DAKO) diluted 1:1500; and ki-67 (MiB-1) (Zytomed, Berlin, Germany) diluted 1:150.

We identified the adenomas by their architecture which was sinusoidal, diffuse or solid in contrast to the alveolar basic structure of the normal pituitary and by their nearly or completely monocellular composition. Focal hyperplasias were not encountered in this study. They were differentiated from the adenomas by their nearly intact alveolar basic structure.

The available clinical data were rescreened from the files for any information about hypertension, diabetes mellitus, hypo- and hyper-thyroidism and any other endocrinological disorders.

Statistical analysis

For statistical analysis, Mann–Whitney test and Fisher's exact test were used for comparing tumour size, gender, adenoma type and clinical data. Statistical significance was established at the level of $P < 0.05$.

Results

A total of 334 pituitary adenomas in 316 of 3048 pituitaries (10.4%) were found. The cases included

157 male and 159 female patients in the age range of 28–97 years (mean 73 years).

The most frequently detected tumours were sparsely granulated PRL cell adenomas (132 cases, 39.5%). The results of the morphological tumour type are summarized in Table 1. A total of 17 pituitaries comprised multiple tumours, 16 cases included two tumours and one case three adenomas (Table 2).

GH cell adenomas

In seven cases, tumour cells exhibited immunopositivity for GH. Tumour size varied from 0.75 to 8.8 mm (median 2.3 mm, Table 3). Four sparsely granulated GH cell adenomas were composed of monomorphic medium-sized tumour cells with chromophobic or weakly acidophilic cytoplasm. Fibrous bodies were demonstrated by globular expression of Pankeratin KL 1. Three densely granulated tumours exhibited a distinct acidophilic cytoplasm.

Table 2 Multiple adenomas.

Adenoma type		<i>n</i>
Largest tumour	2nd/3rd tumour	
PRL cell adenoma	PRL cell adenoma	3
PRL cell adenoma	Null cell adenoma	4
Null cell adenoma	Null cell adenoma	3
Oncocytoma	Null cell adenoma	2
ACTH cell adenoma, densely granulated	Null cell adenoma	1
ACTH cell adenoma, densely granulated	PRL cell adenoma	2
ACTH cell adenoma, sparsely granulated	Oncocytoma	1
TSHoma	Two null cell adenomas	1
Total		17

Table 3

Adenoma type	n	Adenoma size (mm)			
		Min.	Max.	Mean	Median
PRL cell adenoma	132	0.1	6.0	1.64	1.2
ACTH cell adenoma	46	0.15	5.0	1.78	1.4
FSH/LH cell adenoma	22	0.15	20.0	3.58	1.55
α -Subunit-only adenoma	2	0.8	2.0	1.4	1.7
Null cell adenoma	75	0.1	6.0	1.54	1.3
Oncocytoma	31	0.5	7.0	2.62	2.0
Crooke's cell adenoma	1	3.0	3.0	3.0	3.0
GH cell adenoma	7	0.75	8.8	3.29	2.3
Mixed GH cell–PRL cell adenoma	1	2.0	2.0	2.0	2.0
TSH cell adenoma	2	0.8	1.0	0.9	0.9
Unclassified	6	0.14	1.8	0.89	0.85
Plurihormonal adenoma	9	0.4	10.1	3.1	2.0
Total	334	0.1	20	1.97	1.5

PRL cell adenomas

One hundred and thirty-two (39.5%) of the detected adenomas were immunopositive for prolactin, ranging in size from 0.1 to 6 mm (median 1.2 mm). The adenomas consisted of medium-sized slightly pleomorphic tumour cells with chromophobic or weakly acidophilic often elongated cytoplasm and were classified as sparsely granulated PRL cell adenomas.

Mixed GH/PRL cell adenoma

One tumour measuring 2 mm in diameter was immunopositive for both GH and prolactin, and was classified as sparsely granulated mixed GH/PRL cell adenoma.

TSHoma

Only two tumours (0.6%) with a tumour size of 0.8 and 1 mm exhibited exclusive staining of tumour cells for TSH.

ACTH cell adenoma/Crooke's cell adenoma

A total of 47 tumours were counted among ACTH producing adenomas. Tumour size varied from 0.15 to 5 mm in diameter (median 1.4 mm). Twenty-seven of the ACTH producing tumours were composed of uniform tumour cells with slightly dense chromatin and distinct PAS positive basophil cytoplasm corresponding to a densely granulated ACTH cell adenoma. Nineteen adenomas with weakly granulated PAS positive tumour cells were classified as sparsely granulated adenomas. As these cases did not show Crooke's cells in the tumour-free pituitary a distinct hypercortisolism could be excluded (13, 14). There was one case of Crooke's cell adenoma with Crooke's hyaline change within the tumour cells. Twenty-one ACTH cell adenomas (44.7%) exhibited an adjacent ACTH cell hyperplasia.

Gonadotroph cell adenoma

A total of 22 adenomas were classified as gonadotroph cell adenomas, varying in tumour size from 0.15 to 20 mm (median 1.55 mm). Two macroadenomas had tumour sizes of 12 and 20 mm. The adenomas consisted of monomorphic cells with chromophobic cytoplasm and mostly sinusoidal growth pattern. Eleven tumours were positive for FSH and LH and eight cases were positive for FSH. One case showed immunohistochemical staining for LH, one for LH and α -subunit and one for FSH, LH and α -subunit.

α -Subunit-only adenoma

Two adenomas with a tumour size of 0.8 and 2 mm were exclusively positive for the α -subunit of glycoprotein hormones.

Null cell adenoma/oncocytoma

According to their definition, null cell adenomas and oncocytomas were immunonegative for any hormone, but may contain some scattered positive cells. The tumour size ranged from 0.1 to 7 mm (median 1.5 mm). We found 75 null cell adenomas with mostly small cells and 31 oncocytomas consisting of large tumour cells with slightly acidophilic PAS negative cytoplasm.

Plurihormonal adenomas

Plurihormonal adenomas or multihormonal adenomas secreted unrelated hormones not including the combinations GH/PRL/TSH or FSH/LH. The plurihormonal adenomas varied in tumour size from 0.4 to 10.1 mm (median 2 mm), including one macroadenoma measuring 10.1 mm. A total of five tumours showed immunohistochemical staining for GH, PRL and glycoprotein hormones (plurihormonal adenoma type I). Four

Table 4 Plurihormonal adenomas.

Case no.	Plurihormonal adenoma type	GH	PRL	TSH	FSH	LH	α -SU
173	I	X	X	X	—	X	X
174	I	X	X	X	—	X	X
175	I	X	X	X	X	X	X
176	I	X	X	X	X	X	X
177	I	X	X	—	X	X	—
178	II	—	—	X	X	X	X
179	II	—	—	X	X	X	X
180	II	—	—	X	X	X	X
181	II	X	—	—	X	X	X

α -SU: α -subunit.

tumours contained FSH, LH and TSH or GH (plurihormonal adenoma type II) (Table 4).

Unclassified adenomas

Six adenomas were unclassified due to methodical deficiency. The small tumour tissue was not contained in all sections for immunohistochemical staining. Tumour size ranged from 0.14 to 1.8 mm (median 0.7 mm), mean 0.89 mm.

Tumour size

The overall tumour size ranged from 0.1 to 20 mm in diameter (median 1.5 mm, interquartile range, 1.65). One hundred and thirty-eight (41.3%) tumours had a tumour size of 1 mm or less and 76 adenomas (22.7%) had a tumour size of ≥ 3 mm. Only three tumours were macroadenomas corresponding to a tumour size of more than 10 mm (Table 3). There has been no significant correlation between gender and tumour size (Fig. 1) or adenoma type and corresponding tumour size (Fig. 2).

Figure 1 Gender and tumour size. Only the largest tumour is counted among the 17 cases of multiple adenomas.

Clinical data

Clinical data concerning hypertension, diabetes mellitus and other endocrinological symptoms were collected (Table 5). No symptoms of adeno-hypophyseal hormone hypersecretion were reported. Ninety-nine patients suffered from hypertension and 65 patients were reported to have diabetes mellitus. Cases of hyper- and hypo-thyroidism were quite rare (six and four cases respectively), but none of them exhibited a TSH cell adenoma. There was a significantly higher incidence of diabetes mellitus in the group of 'other' adenomas (TSH cell adenomas, plurihormonal adenomas without GH secretion and unclassified adenomas) (Fisher's exact test, $P=0.007$).

Figure 2 Adenoma type and tumour size. Only the largest tumour is counted among the 17 cases of multiple adenomas. PRL cell adenoma; ACTH, ACTH cell adenoma and Crooke's cell adenoma; FSH, gonadotroph cell adenoma; α -SU, α -subunit only adenoma; Nc, null cell adenoma; Oc, oncocyoma; GH, GH cell adenoma, mixed Gh-PRL-cell adenoma, GH secreting plurihormonal adenoma; Other, TSH cell adenoma, plurihormonal adenoma (without GH secretion), and unclassified adenoma.

Table 5

Adenoma type	Total	Hypertension	Diabetes mellitus	Hyperthyroidism	Hypothyroidism
PRL cell adenoma	126	44	21	2	1
ACTH cell adenoma and Crooke's cell adenoma	46	16	13	2	0
FSH/LH cell adenoma, α -SU-only adenoma, null cell adenoma, oncocytoma	120	30	22	2	3
GH cell adenoma, mixed GH-PRL cell adenoma, GH secreting plurihor- monal adenoma	14	3	3	0	0
Other	10	6	6	0	0
Total	316	99	65	6	4

Other: TSH cell adenoma, plurihormonal adenoma (without GH secretion) and unclassified adenoma. Only the largest tumour is counted in case of multiple adenomas.

Statistical evaluation

No significant association was detectable between tumour size and sex (Mann–Whitney test). Evaluation of the data with Fisher's exact test exhibited a higher incidence of diabetes in the group of other adenomas (TSH cell adenomas, plurihormonal adenomas without GH secretion and unclassified adenomas) ($P=0.007$). No associations between gender and adenoma type, adenoma type and hypertension or adenoma type and tumour size were evident.

Discussion

Incidence of subclinical adenomas showed no sex predominance in accordance with data in studies published previously (2, 6, 15, 16).

In some cases with very tiny adenomas, difficulties to distinguish between small adenomas and nodular hyperplasias were noticeable, but in most cases the loss of normal stromal pattern and acinar growth pattern was obvious.

Most adenomas in the present study were PRL cell adenomas (39.5%). In previously published autopsy studies, PRL cell adenomas also represented the majority of pituitary adenomas and the incidence ranged from about 40 to 50% (1, 3, 9, 17). In most surgical series, the incidence of prolactinomas was lower because of the medical therapeutic option of these tumours (17–19).

A total of 106 (31.7%) adenomas (75 null cell adenomas and 31 oncocytomas) were identified. In the literature, the incidence of null cell adenomas or adenomas without immunohistochemical staining in postmortem pituitaries ranges from 18 to 50% (2, 6, 9, 20).

Forty-seven adenomas (14.1%) were ACTH cell adenomas; one of them was composed of Crooke's cells. Other autopsy studies found lower percentages up to 7.2% (2, 9, 20), whereas ACTH cell adenomas

represent about 15% of adenomas in surgically removed pituitary adenomas (19, 21).

In the current study, 6.6% of adenomas were gonadotroph cell adenomas corresponding to the findings of Abd el-Hamid *et al.* (2).

Only eight tumours (2.3%) revealed positive staining for GH, although previously reported data ranged from 0 to 18% in postmortem pituitaries (2, 6, 9, 20). Considering all surgically removed pituitary adenomas, GH cell adenomas account for 25–30% (22).

TSH cell adenomas represented only 0.6% of all adenomas in the current study.

Plurihormonal or multihormonal adenomas were classified according to the WHO classification (23), including cases secreting unrelated hormones not showing the combinations GH/PRL/TSH or FSH/LH. In the current study, these tumours were quite rare (2.7%).

Seventeen cases (5.4%) exhibited a total of 35 multiple adenomas. Other authors described multiple tumours in 0.9–19.4% (6, 20, 24). Eighteen of thirty-five multiple adenomas were null cell adenomas or oncocytomas. Kontogeorgos *et al.* also reported a lack of immunohistochemical staining in the majority of multiple adenomas in autopsy material (24). In contrast to these findings Sano *et al.* described a predominance of GH producing adenomas in multiple adenomas detected in surgically resected material (25). These differences correspond to the observation that null cell adenomas are slow growing and may be incidentally detected without any symptoms, whereas most GH cell adenomas cause gigantism or acromegaly.

Subclinical pituitary adenomas are predominantly small with a mean tumour size of 1.9 mm in this study; over 30% of the detected adenomas were smaller than 1 mm in diameter. These findings are comparable to the results of Parent *et al.*, who found 62% of adenomas in postmortem pituitaries to be smaller than 3 mm (4) and to a study by Teramoto *et al.*, who found 66% of subclinical adenomas to be 2 mm or smaller (8). We

encountered only three macroadenomas among the 334 adenomas. In previously published studies, subclinically or incidentally detected macroadenomas were also very rare (3,7,26).

Our statistical analysis did not reveal a clear significant correlation between clinical data and adenoma type. This is in accordance with the results of McComb *et al.* (9). There was a higher incidence of diabetes in the group of other adenomas (TSH cell adenomas, plurihormonal adenomas without GH secretion and unclassified adenomas). Interpreting these results, the low number of 'other' adenomas (only 10 cases were included in this group) has to be considered. Information about fertility, libido, bone density, physical activity or emotional status was not available.

From our findings, we conclude that adenomas in postmortem pituitaries are different from those in surgical series according to the proportion of adenoma types, functional properties and biological behaviour.

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