Relationship of each anterior pituitary hormone deficiency to the size of non-functioning pituitary adenoma in the hospitalized patients

Kosuke Mukai, Tetsuhiro Kitamura, Daisuke Tamada, Masahiko Murata, Michio Otsuki and Iichiro Shimomura

Department of Metabolic Medicine, Osaka University Graduate School of Medicine, Suita, Japan

Abstract. Non-functioning pituitary adenoma (NFPA) is often associated with hypopituitarism. Diagnosis of hypopituitarism is important because of its poor prognosis and low quality of life. Among hypopituitarism, it is difficult to diagnose secondary adrenocortical insufficiency and GH deficiency without hormone stimulation test. Therefore, the aim of our study was to identify patients with NFPA who require more careful endocrinological examination. We examined the relationship between NFPA size and the prevalence of each hypopituitarism or the response of each anterior pituitary hormone by insulin tolerance test, LHRH test and TRH test. We studied 63 patients with NFPA admitted for evaluation of pituitary function and surgical indication. They were classified three groups by tumor diameter. The prevalence of GH deficiency, male secondary hypogonadism, secondary hypothyroidism and PRL deficiency were higher in the group of larger tumor diameter (p<0.0001, p<0.05, p<0.05 and p<0.05, respectively). However, that of secondary adrenocortical insufficiency only tended to be higher (p=0.07). In the group with small NFPA (less than 20 mm), the prevalence of secondary adrenocortical insufficiency was 38% although those of GH deficiency, male secondary hypogonadism, secondary hypothyroidism and PRL deficiency were 0%, 0% and 8% and 9%, respectively. Anterior pituitary hormone responses except TSH had significantly negative correlation with tumor diameter (ACTH: r=-0.40, GH: r=-0.57, LH: r=-0.69, FSH: r=-0.46, PRL: r=-0.36). The results suggested physicians should proactively suspect GH deficiency, male secondary hypogonadism and secondary hypothyroidism in patients with larger NFPA. On the other hand, adrenocortical function should be examined even in patients with small NFPA.

Key words: Non-functioning pituitary adenoma, Hypopituitarism, Pituitary tumor diameter, Secondary adrenocortical insufficiency
that the likelihood of hypopituitarism increased in larger NFPA[s] [12], suggesting that the size of pituitary adenoma is a potential marker of hypopituitarism. However, this study defined hypopituitarism as at least one anterior pituitary hormone deficiencies, such as secondary adrenocortical insufficiency, GH deficiency, secondary hypogonadism and secondary hypothyroidism, and did not indicate each deficiency. Therefore, it should be evaluated the relationship between the size of pituitary adenoma and each pituitary hormone deficiency, respectively, in particular secondary adrenocortical insufficiency and secondary hypothyroidism, because these two hormone deficiencies are critical for survival.

The aim of the present study was to identify patients with NFPA who should require more careful endocrinological examination, by elucidating the relationship between NFPA size and prevalence of each pituitary hormone deficiency.

Subjects and Methods

Subjects

Patients’ selection criteria in this study were summarized in Fig. 1. The subjects were recruited from NFPA patients (n=78) previously admitted to Osaka University Hospital between January 2009 and April 2015 for evaluation of pituitary functions and surgical indication. All patients had no symptom such as cushingoid or acromegalic features. They also had no endocrinological data suggesting Cushing disease and acromegaly such as elevated serum or urinary cortisol and serum IGF-1 over the upper limit of normal range [13]. There were no patients of hyperthyroidism. In the patients with hyperprolactinemia, we ruled out prolactinoma based on their serum prolactin level relative to their tumor size [14]. The exclusion criteria were the followings: conditions that could affect pituitary function (primary hyperthyroidism [n=1], steroid use [n=3], levothyroxine sodium use [n=2]), and conditions affecting measurement of pituitary tumor size (history of pituitary surgery [n=9]). After application of the above exclusion criteria, the study subjects included 63 patients. None had pituitary cystic lesion that could possibly affect pituitary function and tumor size. Surgical indication was based on visual field defect, risk of optic nerve compression and tendency to tumor development. Neurosurgeons decided not to perform surgery in 9 of 63 patients. Therefore, these 9 patients were only evaluated pituitary function but not operated.

The study protocol was approved by the Human Ethics Committee of Osaka University (No.15310) and performed according to the Helsinki declaration.

Clinical diagnosis of each pituitary deficiency

Secondary adrenocortical insufficiency and GH deficiency were diagnosed by ITT (secondary adrenocortical insufficiency; peak serum cortisol level: <18 μg/dL, GH deficiency; peak serum GH level: <3 ng/mL) [15-17]. As for secondary hypogonadism, the diagnosis was limited to men because the study included only a few female subjects and the majority was old in age (only 10 subjects were <50 years of age) and had no menstrual information. Male secondary hypogonadism was diagnosed on the basis of total testosterone level.
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below the lower limit of the normal range (below normal limit of normal) [18]. The 11 male subjects with elevated basal LH and FSH were diagnosed as primary hypogonadism and 2 men didn’t have the data of total testosterone. Thus, we evaluated secondary hypogonadism in 29 male patients. Diagnosis of secondary hypothyroidism was based on low serum free T4 level (below normal limit of normal) with inappropriately normal or low TSH level (less than upper limit of normal in our hospital [<3.8 μU/mL]) [19]. PRL deficiency was diagnosed based on relative PRL (peak PRL/basal PRL) by TRH test. Although two cut-off value of relative PRL, 2.5 fold and 2 fold, were referred, PRL deficiency was diagnosed by cut-off value of 2 fold because we got the same conclusion using each cut-off [17, 20]. Hyperprolactinemia (PRL >20 ng/mL) was regarded as preservation of PRL secretion capacity. In general, hypopituitarism was defined as pituitary hormone deficiency with at least one among secondary adrenocortical insufficiency, GH deficiency, secondary hypogonadism and secondary hypothyroidism.

**Evaluation of secretory capacity of each anterior pituitary hormone**

ITT was used to evaluate ACTH and GH secretion. None of the participating patients had high basal plasma ACTH or high basal serum GH. Because ITT have not been performed for patients with the risk for seizure or ischemic attack by hypoglycemia, only 45 patients were evaluated. Hypoglycemia in ITT was defined as blood glucose (BG) level <40 mg/dL (n=37) and/or half of baseline BG level (n=7). Because hypoglycemia by ITT was not induced in one patient, we evaluated 44 patients with respect to secondary adrenocortical insufficiency and GH deficiency. We evaluated the peak ACTH and GH in this test. Thyroid stimulating hormone (TSH)/prolactin (PRL) secretion and luteinizing hormone (LH)/follicular stimulating hormone (FSH) secretion were evaluated by the thyrotropin releasing hormone (TRH) test and luteinizing hormone–releasing hormone (LHRH) test, respectively. Three patients with high basal thyroid stimulating hormone (TSH) were diagnosed as subclinical primary hypothyroidism and excluded. Thus, we evaluated TSH secretion in 52 patients after the exclusion of the above three patients. We evaluated the ΔTSH (peak TSH minus basal TSH) [17, 21]. Basal PRL was also elevated in 14 of the patients performed TRH test, suggesting abnormal regulation through inhibition of the dopamine effect from the hypothalamus. The dysregulation altered the correlation between size and PRL secretion. Thus, we evaluated the relative PRL (peak PRL/basal PRL) [17, 20]. None of the female subjects were assessed for LH and FSH secretion based on the reasons mentioned above. The 11 male subjects with elevated basal LH and FSH were diagnosed as primary hypogonadism and 4 men were not examined with LHRH test. Thus, we evaluated LH and FSH secretion in 27 male patients. In these patients, we evaluated the relative LH and FSH (peak LH or FSH/basal LH or FSH) [17]. We also analyzed ΔLH and ΔFSH (peak LH or FSH minus basal LH or FSH) and got the same conclusion as relative LH and FSH (data not shown) [22].

Blood samples were obtained before, and 15, 30, 45, 60, 90, 120 minutes after intravenous administration of insulin, 100 μg LHRH or 250 μg TRH.

**Measurement of tumor size, tumor volume, Knosp grade and pituitary stalk angle**

By tracing the tumor, the maximum tumor diameter and tumor volume were measured using an image analysis software (Image J, National Institutes of Health, Bethesda, MD, http://rsbweb.nih.gov/ij/), as described previously [23]. We classified the tumors according to Knosp grade [24] and evaluated the angle between pituitary stalk and craniocaudal axis as ‘pituitary stalk angle’. In 19 patients, this angle could not be detected because of suprasellar tumor development.

**Evaluation of visual field defect**

Visual field was evaluated by Goldmann perimeter. One patient with 17.5 mm NFPA was excluded from the subjects by glaucoma.

**Laboratory tests**

Blood samples in fasting and in stimulation tests were obtained in the morning while the patient rested in supine position. Plasma ACTH levels were measured by electrochemiluminescence Immunoassay (ECLusys ACTH kit, Roche Diagnostics, Tokyo, Japan) with an inter-assay coefficient of variation (CV) of <25% and intra-assay CV of <10%. Serum cortisol levels were measured by chemiluminescent enzyme immunoassay (Access cortisol kit, Beckman Coulter, Tokyo). This kit has a sensitivity of 0.4 μg/dL, with an intra-assay CV of <4.3% and inter-assay CV of <5.9%. Serum GH lev-
els were measured using a chemiluminescent enzyme immunoassay kit (Access hGH kit, Beckman Coulter, Tokyo) with a sensitivity of 0.002 ng/mL and CV values of inter- and intra-assays of <20% and <15%, respectively. Serum LH and FSH levels were determined by chemiluminescent enzyme immunoassay (Access LH kit and Access FSH kit, Beckman Coulter, Tokyo). Both kits had a sensitivity of 0.2 IU/mL, with inter-assay CV of <25% and intra-assay CV of <10%. Serum total testosterone levels were measured by chemiluminescent enzyme immunoassay (Access Testosterone kit, Beckman Coulter, Tokyo), with a sensitivity of 0.1 ng/mL, intra- and inter-assay CV of <5.0%. Serum TSH levels were assessed by immunoenzymometric assay method (TOSOH-II ST AIA-PACK TSH, Tosoh Bioscience Inc.), with intra- and inter-assay CV of <3.9%. Serum FT4 levels were measured by enzyme immunoassay (TOSOH-II ST AIA-PACK FT4, Tosoh Bioscience Inc., Tokyo) with CV values of intra- and inter-assays of <3.9%. Serum PRL was measured by chemiluminescent enzyme immunoassay (SphereLight Prolactin kit, Wako, Tokyo, from January 2006 to May 2012 and Access Prolactin kit, Beckman Coulter, Tokyo, from April 2012 to November 2013). The former kit has a sensitivity of 0.5 ng/mL, with intra-assay CV of <10% and inter-assay CV of <15%, while the latter kit has a sensitivity of 0.25 ng/mL, with intra-assay CV of <1.54% and inter-assay CV of <4.23%. The PRL level (A) measured by the former kit was converted to PRL values (B) for the latter using the formula; B=(0.92×A)+2.1 (r=0.98).

Statistical analysis

Data of clinical characteristics are presented as median with interquartile range (IQR, first and third quartiles). Age, body mass index (BMI) were compared between each group based on tumor diameter by Kruskal-Wallis test and Steel-Dwass test. Sex and prevalence (visual field defect, hypopituitarism and each pituitary hormone deficiency) were analyzed by Cochran-Armitage test.

Because of a few Patients with NFPA ≤9 mm (n=3) and ≥40 mm (n=2), clinical characteristics were compared between patients with tumor diameter of 1-19 mm, 20-29 mm and ≥30 mm. Prevalence according to tumor volume were analyzed by inter-tertile range (first tertile 123-3,308 mm³, second tertile 3,309-6,350 mm³, third tertile 6,351-30,535 mm³). Pituitary stalk angle was sorted by inter-tertile range (first tertile 5.4-28.1 degree, second tertile 28.2-44.9 degree, third tertile 45-69.7 degree). Nineteen patients with undetectable ‘pituitary stalk angle’ were categorized as N.D. group. Prevalence of each anterior pituitary hormone deficiency according to hyperprolactinemia and visual field defect were evaluated by Fisher’s exact test. The cut-off value, sensitivity, and specificity were derived from the receiver operating characteristic (ROC) analysis. Pearson’s correlation coefficient was used to test the correlation between tumor diameter and the results of each hormone during ITT, LHRH test and TRH test. JMP Pro software (ver. 10.0.2 for Window, SAS Institute, Cary, NC) was used for all analyses.

Results

Clinical characteristics

The study included 63 patients (21 females and 42 males) with a median age of 58 years (IQR 47-70), median body mass index (BMI) of 23.6 kg/m² (IQR 21.7-25.5) and median tumor diameter of 25.6 mm (IQR 20.6-31.6). Except one patient with glaucoma, 61% (38/62) of the patients had visual field defect. Headache and general malaise were recorded in 30% (19/63) and 4.8% (3/63) of all patients. Ten percent (3/29) of men suffered with hypogonadal symptoms. All patients except 3 harbored macroadenoma measuring ≥10 mm in diameter. In all study subjects, the prevalence of secondary adrenocortical insufficiency and GH deficiency was 55% (24/44) and 59% (26/44), respectively, among the subjects evaluated by ITT. The prevalence of secondary hypogonadism in men was 41% (12/29). The prevalence of secondary hypothyroidism and PRL deficiency was 30% (18/60) and 24% (14/58), respectively, among the subjects evaluated by ITT. The prevalence of secondary hypopituitarism in men was 41% (12/29). The prevalence of secondary hypothyroidism and PRL deficiency was 30% (18/60) and 24% (14/58), respectively. The prevalence of hypopituitarism with at least one anterior pituitary hormone deficiency was 64% (40/63). The clinical characteristics by groups based on NFPA size are summarized in Table 1. Sex and BMI were not significantly different according to tumor diameter. The group of tumor diameter smaller than 20 mm was younger than the group of 20-29 mm tumor (p<0.05). Prevalence of visual field defect was significantly increased in the group of larger NFPA (p<0.0001). The group of larger NFPA have significantly higher prevalence of hypopituitarism (with at least one pituitary hormone deficiency, p<0.01). In addition, the group of larger NFPA was associated with more pituitary hormone deficiencies (p<0.005, data not shown).
Prevalence of hypopituitarism according to tumor diameter

Significant high prevalence of GH deficiency, male secondary hypogonadism, secondary hypothyroidism and PRL deficiency in the group of larger tumor diameter were demonstrated (Fig. 2, \(p<0.0001, p<0.05, p<0.05, \) and \(p<0.05, \) respectively). Among patients with NFPA smaller than 20 mm, none of them was diagnosed as GH deficiency and male secondary hypogonadism. Only one of them was diagnosed as secondary hypothyroidism. The sensitivity, specificity and optimal cut-off value of tumor diameter were 88.9\%, 73.1\% and 25.9 mm, respectively, for GH deficiency (AUC 0.888), 92.3\%, 75.0\% and 24.1 mm, respectively, for male secondary hypogonadism (AUC 0.817), 83.3\%, 47.7\% and 24.1 mm, respectively, for secondary hypothyroidism (AUC 0.654) and 71.4\%, 72.7\% and 29.4 mm, respectively, for PRL deficiency (AUC 0.693). The cut-off value not to miss the patient with GH deficiency, male sec-

Table 1 Clinical characteristics of participating patients according to tumor diameter

| Tumor diameter (mm) | 1-19 | 20-29 | ≥30 | \(p\) value |
|---------------------|------|-------|-----|------------|
| Sex (F/M)           | 3/10 | 10/18 | 8/14 | NS         |
| Age (year)          | [49 (38.5-54.5)] | [64.5 (56.0-70.8)] | [56.5 (46.5-72.3)] | \(\star p<0.05\) |
| BMI (kg/m\(^2\))    | [24.1 (22.1-25.9)] | [23.3 (21.4-24.6)] | [24.2 (21.4-27.2)] | NS         |
| Visual field defect | 8\%  | 54\%  | 100\% | \(p<0.0001\) |
| Hypopituitarism     | 38\% | 61\%  | 82\% | \(p<0.01\)  |

Data are median values (1st and 3rd quartiles) or percentage of patients (affected/all evaluated patients). BMI, body mass index; NS, not significant. Hypopituitarism represent at least one anterior pituitary hormone deficiency such as secondary adrenocortical deficiency, growth hormone deficiency, secondary hypogonadism and secondary hypothyroidism. A patient with 17.5 mm NFPA were excluded from subjects of visual defect by glaucoma. \(\star\) With respect to age, the group of tumor diameter smaller than 20 mm was younger than the group of 20-29 mm tumor (\(p<0.05\)).

Fig. 2 Prevalence of hypopituitarism in NFPA patients with different tumor size (secondary adrenocortical insufficiency [white bar]: \(n=44\), GH deficiency [gray bar]: \(n=44\), male secondary hypogonadism [black bar]: \(n=29\), secondary hypothyroidism [dotted bar]: \(n=60\), PRL deficiency [horizontal-striped bar]: \(n=58\) ).

Data are percentage of patients and affected/all evaluated patients. Significant high prevalence of GH deficiency, male secondary hypogonadism, secondary hypothyroidism and PRL deficiency in the group of larger tumor diameter were demonstrated by Cochran-Armitage test (\(p<0.0001, p<0.05, p<0.05\) and \(p<0.05, \) respectively).
ondary hypogonadism, secondary hypothyroidism and PRL deficiency was 20.6 mm (sensitivity 100%, specificity 50.0%), 21.1 mm (sensitivity 100%, specificity 62.5%), 19.3 mm (sensitivity 100%, specificity 28.5%) and 16.9 mm (sensitivity 100%, specificity 18.1%), respectively. In contrast, prevalence of secondary adrenocortical insufficiency tended to be increased in the group of larger NFPA but did not significantly depend on tumor diameter \((p=0.07)\). Thirty-eight percent of patients with NFPA smaller than 20 mm were diagnosed as secondary adrenocortical insufficiency. The sensitivity, specificity and optimal cut-off value of tumor diameter were 66.7%, 75.0% and 25.9 mm, respectively, for secondary adrenocortical insufficiency \(\text{AUC} 0.666\). The cut-off value not to miss the patient with secondary adrenocortical insufficiency was 9.0 mm (sensitivity 100%, specificity 0%).

**Prevalence of hypopituitarism according to other clinical features and image parameters**

Significant high prevalence of GH deficiency, secondary hypothyroidism and PRL deficiency in NFPA group of larger tumor volume were demonstrated \((p<0.0001, p<0.05 \text{ and } p<0.05, \text{ respectively})\). Lateral tumor development (Knosp grade) was related with GH deficiency and male secondary hypogonadism \((p<0.01 \text{ and } p<0.01, \text{ respectively})\). Pituitary stalk angle was only related with GH deficiency \((p<0.01)\). Hyperprolactinemia had no relationship with any pituitary hormone deficiency \((p=1.00)\). Visual field defect was only related to GH deficiency \((p<0.005)\). Secondary adrenocortical insufficiency was not significantly related with any clinical features and image parameters \((r=-0.20, \text{ Knosp grade: } p=0.20, \text{ pituitary stalk angle: } p=0.29, \text{ hyperprolactinemia: } p=1.00, \text{ visual field defect: } p=0.33\) \(\text{Figs. 3, 4}\).

**Secretory capacity of pituitary hormone according to tumor diameter**

Fig. 5 shows the correlation between pituitary hormones secretion capacity and tumor diameter. Peak plasma ACTH and serum GH in the ITT \(n=44\), relative serum LH and serum FSH in the LHRH test \(n=27\) men, and relative serum PRL in the TRH test \(n=43\) correlated negatively with tumor diameter \(\text{ACTH: } r=-0.40, p<0.01, \text{ GH: } r=-0.57, p<0.0001, \text{ LH: } r=-0.69, p<0.0001, \text{ FSH: } r=-0.46, p<0.05, \text{ PRL: } r=-0.36, p<0.05\). In contrast, there was no correlation between ΔTSH and tumor diameter \(n=52, r=0.52\). ΔTSH related negatively with peak cortisol in the ITT \(n=41, r=-0.39, p<0.05\) \(\text{Fig. 6}\).

**Discussion**

In the present study, prevalence of GH deficiency, male secondary hypogonadism, secondary hypothyroidism and PRL deficiency were significantly higher in NFPA group of larger tumor diameter \(\text{Fig. 2: } p<0.0001, p<0.05, p<0.05 \text{ and } p<0.05, \text{ respectively})\). However, that of secondary adrenocortical insufficiency only tended to be higher without statistical significance by the diameter of NFPA \(\text{Fig. 2: } p=0.07)\). We also demonstrated the correlation between the secretion capacity of each anterior pituitary hormone and tumor diameter, based on the hormone stimulating tests \(\text{Fig. 5, ACTH: } r=-0.40, p<0.01, \text{ GH: } r=-0.57, p<0.0001, \text{ LH: } r=-0.69, p<0.0001, \text{ FSH: } r=-0.46, p<0.05, \text{ PRL: } r=-0.36, p<0.05\). Our study analyzed each anterior pituitary hormone deficiency according to NFPA size for the first time. Differently from a previous study that analyzed the prevalence of hypopituitarism in NFPA \(\text{[12]}\), we appropriately confirmed the diagnosis of secondary adrenocortical insufficiency and GH deficiency by ITT, which should be the golden standard method for diagnosis.

Prevalence of secondary adrenocortical insufficiency only tended to be increased in the group of larger tumor diameter although peak plasma ACTH in the ITT was correlated with tumor diameter. Moreover, as much as 38% of patient were diagnosed as secondary adrenocortical insufficiency among patients with NFPA smaller than 20 mm. In addition, the specificity was 0% when we set the cut-off value not to miss the patient with secondary adrenocortical insufficiency \(\text{the cut-off value of NFPA size: } 9.0 \text{ mm}\). This result indicated that it was difficult to identify secondary adrenocortical insufficiency by NFPA size. Hypothalamic-pituitary-adrenocortical axis plays an important role for survival, so missing diagnosis of secondary adrenocortical insufficiency should be avoided. Our results suggested that ITT should be performed even in patients with small NFPA to the diagnosis of secondary adrenocortical insufficiency.

On the other hand, all with NFPA smaller than 20 mm reached peak cortisol levels more than 15 μg/dL although the levels did not fulfill the diagnostic cut-
Adrenocortical dysfunction in small NFPA

Fig. 3 Relationship between pituitary tumor image and prevalence of secondary adrenocortical insufficiency (white bar, n=44), GH deficiency (gray bar, n=44), male secondary hypogonadism (black bar, n=29), secondary hypothyroidism (dotted bar, n=60) and PRL deficiency (horizontal-striped bar, n=58)

The trends of prevalence according to pituitary tumor image were analyzed by Cochran-Armitage test. (A) Significant high prevalence of GH deficiency, secondary hypothyroidism, PRL deficiency in NFPA group of larger tumor volume (p<0.0001, p<0.05 and p<0.05, respectively). 1: first tertile 123-3,308 mm³, 2: second tertile 3,309-6,350 mm³, 3: third tertile 6,351-30,535 mm³. (B) Significant high prevalence of GH deficiency and male secondary hypogonadism in NFPA group with more lateral tumor development (Knosp grade) (p<0.01 and p<0.01, respectively). (C) Significant high prevalence of GH deficiency with larger pituitary stalk angle (p<0.01). 1: first tertile 5.4-28.1 degree, 2: second tertile 28.2-44.9 degree, 3: third tertile 45-69.7 degree. ND: not detectable pituitary stalk angle.
off of secondary adrenocortical insufficiency (peak cortisol ≥18 μg/dL). A previous study on prolactinoma but not NFPA showed that the group of larger tumor had significantly high prevalence of secondary adrenocortical insufficiency which was defined as peak cortisol after ACTH stimulation test <16.3 μg/dL (450 nmol/L) [25]. Similarly in this study, the proportion of subjects with cortisol peak less than 15 μg/dL was significantly high in the group of larger NFPA (Supplemental Fig. 1: p<0.05). This result corresponded to negative correlation between peak plasma ACTH and tumor diameter. Thus, secondary adrenocortical insufficiency associated with small NFPA might be considered as mild secondary adrenocortical insufficiency whose peak cortisol was under 18 μg/dL but over 15 μg/dL.

![Graph A](image1)

**Fig. 4** Difference in prevalence of anterior pituitary hormone deficiency according to hyperprolactinemia and visual field defect (A) Hyperprolactinemia had no relationship with any pituitary hormone deficiency (Secondary adrenocortical insufficiency: n=44, p=1.00, GH deficiency: n=44, p=1.00, male secondary hypogonadism: n=29, p=0.63, secondary hypothyroidism: n=60, p=0.32). Non-hyperprolactinemia: white bar, hyperprolactinemia: left hatched bar. (B) Visual field defect was related to GH deficiency (Secondary adrenocortical insufficiency: n=44, p=0.33, GH deficiency: n=44, p<0.005, male secondary hypogonadism: n=28, p=0.34, secondary hypothyroidism: n=59, p=0.15, PRL deficiency: n=57, p=0.11). Normal visual field: white bar, visual field defect: right hatched bar.
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Fig. 5 Correlations between tumor diameter and response of pituitary hormone in the insulin tolerance test (ITT), LHRH test and TRH test. Regression line: the best fit for the relation between the two variables. (A) Tumor diameter and peak ACTH level in ITT (n=44, \( r=-0.40 \), \( p<0.01 \)). (B) Tumor diameter and peak GH level in ITT (n=44, \( r=-0.57 \), \( p<0.0005 \)). (C), (D) Tumor diameter and relative level of LH and FSH in the LHRH test (n=27 men, LH: \( r=-0.69 \), \( p<0.0001 \), FSH: \( r=-0.46 \), \( p<0.05 \)). (E) Tumor diameter and ΔTSH in the TRH test (n=52, \( p=0.52 \)). (F) Tumor diameter and relative PRL level in the TRH test (n=43, \( r=-0.36 \), \( p<0.05 \)).
In respect of GH and LH/FSH, there was negative correlation between their secretion capacity and tumor diameter. Correspondingly, we also confirmed a significantly high prevalence of GH deficiency and male secondary hypogonadism in the group of larger NFPA. These results indicated hypopituitarism of these axes was byproduct of tumor mass effect. Among patients with NFPA smaller than 20 mm, none of them was diagnosed as GH deficiency and male secondary hypogonadism. In addition, the cut-off value of tumor diameter not to miss the patient with GH deficiency and male secondary hypogonadism was about 20 mm. The findings might lead us to a proactive examination of GH deficiency and secondary hypogonadism in NFPA larger than 20 mm. This would be useful not only at first image of pituitary in all NFPA patients but also at follow-up one in case of NFPA patients who were observed without pituitary operation.

The present study also showed that the prevalence of secondary hypothyroidism significantly increased in the group of larger NFPA. Only one patient, whose tumor diameter was 19.3 mm, was diagnosed as GH deficiency and male secondary hypogonadism among patients with NFPA smaller than 20 mm. Thus, the cut-off value of tumor diameter not to miss the patient with secondary hypothyroidism was almost 20 mm. Therefore, NFPA larger than 20 mm might be proactively examined for secondary hypothyroidism. Differently from GH and LH/FSH secretion capacity, there was no correlation between ΔTSH and tumor diameter. On the other hand, ΔTSH correlated negatively with peak cortisol (Fig. 6). Previous studies reported an important role of glucocorticoid in TSH secretion [26, 27]. One previous study found high TSH levels in Addison’s disease and that the levels returned to normal after replacement of glucocorticoids [28]. TSH response to TRH might be influenced by adrenocortical functional status, which might be the reason why there was the discrepancy between prevalence of secondary hypothyroidism and TSH response according to tumor diameter.

We also analyzed the prevalence of each anterior pituitary hormone deficiency with clinical features and image parameters in addition to tumor diameter (Fig 3, 4). Similar to tumor diameter, tumor volume related with prevalence of anterior pituitary hormone deficiency except male secondary hypogonadism. In our patients, there were strong correlation between tumor diameters and volumes ($r=0.78$, $p<0.0001$, data not shown). Theoretically, the tumor volumes but not the tumor diameter is more proper considering that tumor progression is three-dimensional. However our study investigated mainly the relation between tumor diameter and prevalence of each anterior pituitary deficiency because the tumor diameter is easier to measure in clinical practice. On the other hand, other parameters were related with only a few anterior pituitary deficiencies. Knosp grade, hyperprolactinemia, visual field defect and pituitary stalk angle were associated with suprasellar or lateral tumor progression. These features and parameters described above might not fully reflect the damage of anterior pituitary gland because these parameters evaluated tumor position but not the position of anterior pituitary gland. All of our clinical features and image parameters had no significant relation with secondary adrenocortical insufficiency. It was suggested that this insufficiency was not predictable based on our clinical features or image parameters. Therefore, ITT should be performed for the exact diagnosis.

The prevalence of hypopituitarism in our study, which was defined as at least one anterior pituitary deficiency, was less than those reported in previous studies (69-89%) [12, 29]. It is possible that the difference is due to differences in the methods used for evaluation of hypopituitarism. For example, we diagnosed secondary adrenocortical insufficiency or GH deficiency by ITT, the golden standard test, while previous studies used various other methods including basal serum cortisol level, CRH test and ACTH test or low serum IGF-1 level. However, the prevalence of hypopituita-

![Fig. 6 Correlations between serum peak cortisol during insulin tolerance test (ITT) and ΔTSH in the TRH test. Regression line: the best fit for the relation between the two variables ($n=41$, $r=0.39$, $p<0.05$).](image-url)
Adrenocortical dysfunction in small NFPA

Adrenocortical dysfunction in small NFPA in our study was significantly lower in patients with smaller NFPA, in agreement with the finding of a previous study [12].

The present study has certain limitations. We did not investigate secondary hypogonadism in women in the present study due to the lack of information on the menstrual state for all patients and the small number of premenopausal women. Further studies are necessary to determine the relation between female secondary hypogonadism and NFPA size. Our study subjects who have been recruited for evaluation of pituitary functions and surgical indication were another limitations. It was reported that patients with microadenoma are rarely evaluated in terms of hypopituitarism [30]. Accordingly, the number of microadenomas was small in this study (only three patients). In addition, as the limitation of recruitment, the group of tumor diameter smaller than 20 mm was younger than the group of 20-29 mm tumor (Table 1). Because younger patients have long lifetime than older patients, the former has more probability of tumor progression. Therefore, pituitary surgery might be recommended for the former [30]. Also, there are significant risks of surgical procedure in older patients. These might affect recruitment of the subjects. Although there were these limitations, the correlation between GH, LH/FSH secretion capacity and tumor diameter, that is, the etiology of mass effect mentioned above, may suggest less prevalence of GH deficiency and secondary hypogonadism in pituitary microadenoma.

In conclusion, physicians should proactively examine pituitary function in patients with NFPA measuring ≥20 mm, in particular, GH deficiency, male secondary hypogonadism and secondary hypothyroidism. Differently from GH deficiency, male secondary hypogonadism and secondary hypothyroidism, secondary adrenocortical insufficiency did not significantly correlate with NFPA size. Our results also suggest that examination of adrenocortical function is warranted even in patients with small NFPA.

Disclosure

The authors declare no conflict of interest.

Supplemental Fig. 1 Proportion of NFPA patients with peak cortisol less than 15 μg/dL. Data are percentage of patients and affected/all evaluated patients (n=44). Significantly higher proportion in the group of larger NFPA were demonstrated by Cochran-Armitage test (p<0.05).

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