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SERUM LEVELS OF FSH AND TSH IN NONFUNCTIONING PITUITARY ADENOMAS BEFORE AND AFTER SURGERY

SABA KHALID, KHALID MAHMOOD AND M. ARSLAN
Department of Physiology and Cell Biology, University of Health Sciences, Lahore

ABSTRACT

Clinically nonfunctioning human pituitary adenomas (NFPAs) constitute about 25-35% of pituitary tumours. Relatively few studies have been carried out to determine the cellular origin and biology of the NFPAs. The present study was undertaken to assess the effect of surgical removal of adenoma on peripheral adenohypophyseal hormones. In addition, the pituitary hormones have also been measured directly in the adenoma tissue following its surgical removal. The present study is based on 19 patients (16 males and 3 females), 30-50 year of age, diagnosed for NFPAs on the basis of clinical and radiological evidence. Follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) were determined in serum before and following transsphenoidal adenomectomy. The FSH, TSH content of the excised adenoma tissue was also measured in male patients. The results demonstrate that in a subset of 4 of the 16 male patients with NFPAs the presurgical serum FSH values (45.1±0.7 mIU/ml) were markedly and significantly greater than those of the remaining 12 patients (7.9±0.7 mIU/ml) and of control subjects (8.8±0.6 mIU/ml). Following removal of the adenoma in these 4 patients, peripheral serum FSH levels fell within the normal range. In the remaining 12 patients peripheral and mean FSH levels were within the normal range. Following removal of the adenoma in 2 female patients, serum FSH concentrations showed a marked decline of FSH levels (5.7 mIU/ml and 7.2 mIU/ml) whereas in the remaining one patient, the FSH levels remained relatively high (34.5 mIU/ml). The adenoma tissue FSH content in patients with initial higher serum FSH levels, was also significantly greater than that of the other patients (21.5±2.1 vs 4.4±1.9 IU/g). The presurgical mean serum TSH concentrations in patients with NFPA were mostly in the lower normal range and were not significantly different from the control (1.0±0.2 vs 1.5±0.4 µIU/ml) and removal of the adenoma did not affect the serum TSH levels significantly. The TSH content of adenoma tissue was either non-detectable or in the low range as compared to the TSH content determined in control pooled pituitary tissue. The present study suggests that pituitary tumours diagnosed as NFPAs constitute a heterogenous group of adenomas with regard to their secretory activity of intact adenohypophyseal hormones. Whereas some of these adenomas activity may synthesize one or more pituitary hormones, peripheral concentrations may not be sufficient to produce overt clinical symptoms of hormone hypersecretion.

Key words: *FSH-secreting adenoma, TSH-secreting adenoma, Pituitary content, Glycoprotein-secreting adenomas.*

INTRODUCTION

Pituitary tumours are abnormal growths of the pituitary gland that may secrete excessive amounts of hormones and also in some instances, restrict the pituitary gland resulting in lower peripheral levels of hormone secretion. In most cases, pituitary tumours remain confined to pituitary gland and the associated tissues. Since these tumours do not spread to other parts of the body, therefore, the term adenomas is generally used to designate these growths.[1,2]

Pituitary adenomas are usually well demarcated and are seperated from the adjacent compressed nontumorous adenohypophysis by a pseudocapsule that consists of condensed reticulin fibres.[2,3] These adenomas may be classified according to their size, radiographical appearance, endocrine functions, morphology and cytogenesis. Clinicians frequently classify pituitary adenomas on the basis of their size as determined by imaging studies. Those smaller than 1 cm are designated as microadenomas and larger than 1 cm are categorized as macroadenomas. Microadenomas are also classified as grade 0 or grade 1 tumours. Macroadenomas that may also cause diffuse sellar enlargement, and extensive sellar destruction and erosion, are classified as grade 2-4.[2,4-9]

Partial or complete hypopituitarism has also been demonstrated in patients with large clinically non-functioning tumours because of compression

of the adjacent normal pituitary tissue. Visual symptoms are found in 60-70% of cases and may be in the form of visual blurring, a loss of temporal field in one or both eyes and loss of visual acuity. Headache is observed in about 40% of patients and is often non-specific dull ache over vertex that does not vary with position or the time of the day.[10-14] There may also be other symptoms including loss of libido in men, amenorrhea, sexual disinterest, presence of smooth pale skin, and chronic fatigue. These clinical findings are supported by peripheral hormone levels that indicate significant pituitary insufficiency.[15,16]

More than 20% of all pituitary adenomas fall in the nonfunctional category, the predominant types being the 'null cell' adenomas or gonadotrope adenomas and 'silent' corticotrope adenomas.[17] Recent advances in immunoassay and immunocytochemical techniques have allowed a more specific characterization of the secretory activity of the non-functional pituitary adenomas (NFPAs). These nonfunctioning tumours invariably contain cytoplasmic secretory granules, suggesting that they do produce specific hormones, biologically inactive precursors, or hormone fragments. Some of these hormonally silent tumours are revealed by immunohistochemistry to contain hormones, but appear to be incapable of discharging these hormones in sufficient quantities to disturb the endocrine equilibrium.[17,18]

Pituitary adenomas that arise from glycoprotein secreting cells especially gonadotrope cells, appear to account for most pituitary macro-adenomas clinically declared as non-functioning. The biological origin of these tumours has not been easy to elucidate but in many cases they appear to be monoclonal in origin. Studies of excised adenoma tissue show that at least two thirds of these adenomas secrete or stain immunospecifically for some combination of intact gonadotrophins and their subunits or express messenger RNAs for gonadotropin subunits[18,19]

There is some evidence that the release of the adenoma secretion in circulation is variably restricted and the levels of hormones determined in the blood may not necessary reflect the hormone synthetic activity of such tumour cells.[20] Relatively high serum FSH with low LH levels, is often the only sign of the secretory activity of such adenomas and predominantly LH-secreting adenomas are rarely seen.[19] On the other hand, serum levels of FSH have been reported as increased in up to 15% of patients with NFPA.[20,21] However, in many patients with these glycoprotein hormone adenomas, serum levels of FSH, LH and the free α- and β-subunits stay within the normal range.[22,23]

A few studies have been carried out to systematically assess the effect of removal of non-functional pituitary adenoma on peripheral adeno-hypophyseal hormones and the correlation of the serum levels with the hormone content of the adenoma tissue. The present study was, therefore, undertaken to determine serum gonadotropin FSH, TSH concentration in patients before and following removal of pituitary adenomas diagnosed presurgically, as non-functional. In addition we have measured hormones content of the adenoma tissues for a possible correlation with serum levels of these hormones.

MATERIALS AND METHODS

Study population

The present study initially included 22 patients 18 men and 4 women, of 22-55 (44 ± 2.3) years of age with NFPAs. Three of these patients 2 men and 1woman died during the follow-up period and were excluded from the study. All patients underwent pituitary tumour surgery. The adenoma was removed in 18 patients through trans-sphenoidal route whereas in one female patient (PA 16) transcranial approach was used. All cases were operated at the Department of Neurosurgery, Lahore General Hospital, Lahore.

Patients with NFPAs were diagnosed on the basis of the following criteria:

a) Presence of a sellar mass with or without extrasellar extension detected by magnetic resonance imaging (MRI) or high resolution computed topographic (CT) scans.
b) Absence of signs or symptoms of functioning pituitary adenoma (acromegaly, Cushing's disease, prolactinomas and hyperthyroidism).
c) Patients presenting signs and symptoms of a sellar mass such as headache and visual problems.
d) Histological confirmation by light microscopy of pituitary tumour in the excised adenoma tissue.

The study also included 22 sex and age matched healthy subjects that served as the control group with 22-55 years of age. Subjects included in the control group had no medical history of any chronic disease and endocrine-pathies, and were not on current or past medication of steroids, anti-psychotropic and other medications known to affect pituitary hormone secretion. The subjects fulfilling inclusion criteria were enrolled in the study after obtaining his/her written informed consent.

Sample collection

Blood samples from patients with pituitary adenomas were obtained prior to surgery and 2 months after removal of the pituitary adenoma. Fol-

lowing surgery, patients received 10 mg prednisolone daily for 6 weeks. Postsurgical serum samples were obtained 2 weeks after the prednisolone treatment was discontinued. Blood samples were also obtained from an equal number of age and sex matched control subjects for purposes of comparison. Five ml of blood sample were drawn from each patient and blood was centrifuged at 3,000 rpm for 10-15 minutes to separate serum and aliquoted in two portions and stored at -30°C until analyzed.

The pituitary adenoma tissue was divided into two parts. One part was fixed in buffered formalin and processed for histological examination, whereas the other half was weighed and homogenized in icecold phosphate buffer saline (PBS), at a dilution of approximately 50 mg/ml. The samples were centrifuged for 30 min at 10000 rpm and the supernatant was frozen at -30°C. Pituitary tissue was also obtained from three adult males at the postmortem examination, pooled and processed identically as the adenoma tissue for extraction. Pituitary tissue from female controls could not be made available.

Tissue processing

Following fixation, adenoma tissue was processed for histopathological examinations. Paraffin sections of the tissue were cut at a thickness of 3-5µm and stained routinely with haemotoxylin and eosin.

Hormone determinations

Serum FSH, TSH were determined by ELISA in duplicate using standard procedures with comercially available assay kits (FSH and TSH: Monobind Inc, Lake Forest, CA ,USA) with an automated EIA analyzer (Coda, Bio-Rad Laboratories, Hercules, CA, USA).Hormone content of adenoma and pituitary tissue, was measured with a specific radiometric assay in the extract obtained, by commercially available IRMA kits (IRMA; Immunoteck, Prague, Republic Czech with an automated gamma counter (Perkin Elmer, Turku, Finland).

Statistical Analysis

The significance of differences among different groups was analyzed by one way analysis of variance {ANOVA} followed by Duncan's multiple t-test. P value of <0.05 was considered statistically significant. All calculations were carried out with the SPSS version 12 (SPSS Inc, Chicago, IL, USA).

RESULTS

Follicle Stimulating Hormone (FSH)

Males

The mean serum FSH levels in the 16 male patients with NFPAs were found to be significantly higher (P<0.05) before surgical removal as compared to those obtained following surgery (17.2 ± 4.2 vs 8.5 ± 1.2 mIU/ml) (Table 1 and Fig. 1). However, an examination of individual values reveals that the observed higher mean levels of FSH prior to surgery, were mainly due to the inclusion of 4 patients (PA10, 26, 29 and 30; Table 2) in which serum FSH levels were almost 4-fold of the control levels (> 40mIU/ml) as shown in Tables 2, 3 and Fig 2. In these subjects serum FSH concentrations returned to the normal range following removal of the adenoma. The adenoma tissue content of FSH of these two subsets of patients is given in Table 3. As may be noticed, the adenoma FSH content in

Table 1: *Mean ± SEM serum FSH levels and FSH content of adenoma tissue, in male patients with NFPA and normal subjects.*

Group	Serum FSH (mIU/ml)	Adenoma tissue FSH content (IU/g)
Control subjects (n = 16)	8.8 ± 0.6	
Patients with pituitary adenoma (n = 16)		8.7± 2.4
Presurgical	17.2 ± 4.2*	
Postsurgical	8.5 ± 1.2	
Pooled pituitary tissue		15.5

*Significantly different from postsurgical and control value (P < 0.05; ANOVA followed by Duncan's t- test).

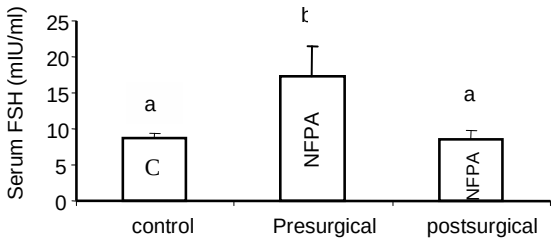

Fig. 1: *Mean ± SEM serum concentration of FSH (mIU/ml) in 16 male patients with NFPA and age-matched control subjects. The values with different superscripts (a,b) are different from each other (P<0.05; ANOVA followed by Duncan's t-test).*

patients with the initial higher peripheral concentrations, was also markedly greater (P<0.05) than that of the patients with normal or low serum FSH levels and the control pituitary tissue. In the remaining 12 patients the difference in mean serum FSH concentrations between pre-surgical and post-surgical serum values, was not significant (Table 3 and Fig. 3). The FSH pituitary content in patients with relatively low normal serum FSH levels, was also comparable to that of the control pooled pituitary tissue (Table 3).

Females

In all the three premenopausal female patients included in the study, the individual presurgical serum FSH levels were greater than those following surgery (Table 4). The mean value was significantly higher than that of the controls (36.4 ± 6.5 vs 8.4 ± 1.1 mIU/ml, respectively). (Table 5 and Fig. 4). These values exceeded the normal serum FSH concentrations described for healthy women (3.0-22.0 mIU/ml). Following surgical removal of the adenoma, serum FSH concentrations showed a marked decline of FSH levels in 2 patients (PA4 and PA16) whereas in the remaining one patient (PA19), the FSH levels persisted at relatively high (34.5 mIU/ml) level even after removal of the adenoma.

Thyroid Stimulating Hormone (TSH)

Males

The individual and the mean serum TSH levels in the 16 male patients with pituitary adenomas are given in Tables 6, 7 and Fig 5. The presurgical mean serum TSH concentrations in patients with pituitary adenoma were mostly in the lower range and were not significantly different from levels following removal of the adenoma and of the control subjects. The TSH content of adenoma tissue was either in the non-detectable range of the assay or comparable to the control pituitary tissue (Table 7).

Table 2: *Serum FSH levels and FSH content of adenoma tissue in male patients with NFPA and in age-matched control subjects.*

Patients with NFPA						Control subjects		
	Patient ID	Age (y)	FSH (mIU/ml) Presurgical	Postsurgical	FSH Adenoma tissue (IU/g)	Patient ID	Age (y)	Serum Control FSH (mIU/ml)
Males								
	PA1	30	8.2	8.6	4.7	C1	30	8.9
	PA2	35	5.4	5.6	1.0	C2	35	7.1
	PA7	22	6.9	6.3	2.3	C3	22	12.4
	PA8	40	5.0	6.1	1.6	C4	40	7.0
	PA9	55	9.2	7.1	24.7	C5	55	7.0
	PA10	45	44.1	26.1	17.2	C6	45	7.7
	PA12	35	11.5	5.2	1.4	C7	35	8.0
	PA14	50	5.3	7.4	1.6	C8	50	8.6
	PA18	50	12.5	5.3	2.3	C9	50	8.3
	PA21	38	5.6	4.2	ND	C10	38	9.8
	PA22	50	9.2	5.5	ND	C11	50	6.6
	PA24	50	6.4	6.8	9.4	C12	50	6.2
	PA26	45	44.1	9.3	19.1	C13	45	11.8
	PA28	42	9.5	9.8	4.2	C14	42	5.9
	PA29	35	45.1	11.0	22.9	C15	35	13.8
	PA30	45	47.3	12.0	26.8	C16	45	11.8

ND: Non detectable levels

Table 3: *Mean ± SEM serum FSH levels and FSH content of adenoma tissue, in male patients with NFPA and normal subjects.*

Group	Serum FSH (mIU/ml)	Adenoma tissue FSH content (IU/g)
Males		
Control Subjects (n= 16)		8.8 ± 0.6
Patients with pituitary adenoma		
(a) Subset I-with low or normal FSH levels (n=12)		4.4 ± 1.9
Pre-surgical	7.9 ± 0.7	
Postsurgical	6.5 ± 0.4	
(b) Subset II-with elevated FSH levels (n=4)		21.5 ± 2.1
Presurgical	45.1 ± 0.7*	
Postsurgical	14.6 ± 3.8	
Pooled pituitary tissue		15.5

*Significantly different from postsurgical and control value (P< 0.05; ANOVA followed by Duncan's t- test).

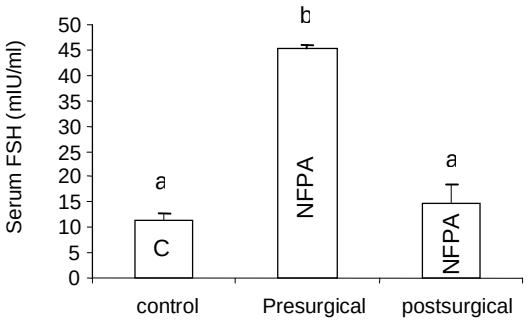

Fig. 2: *Mean ± SEM serum concentration of FSH (mIU/ml) in subset of 4 male patients with high presurgical serum FSH levels and age-matched control subjects. The values with different superscripts (a,b) are different from each other (P<0.05; ANOVA followed by Duncan's t- test).*

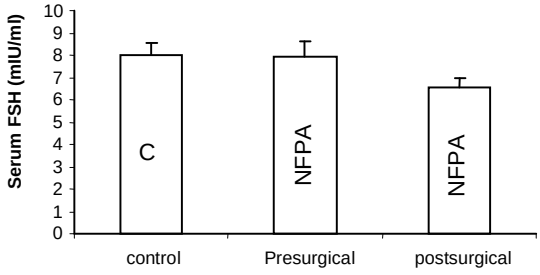

Fig. 3: *Mean ± SEM serum concentration of FSH (mIU/ml) in a subset of 12 male patients with normal serum FSH levels with NFPA and age-matched control subjects.*

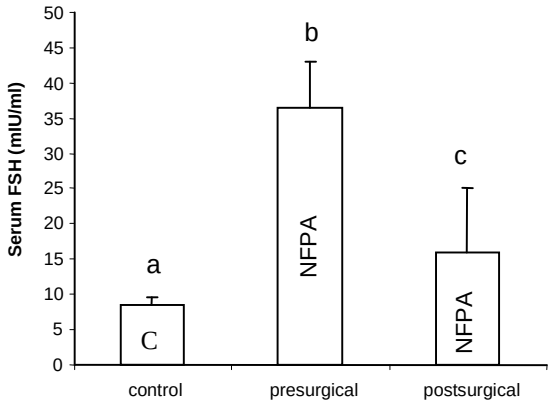

Fig. 4: *Mean ± SEM serum concentration of FSH (mIU/ml) in female patients with NFPA and age-matched control subjects. The values with different superscripts (a,b,c) are different from each other (P<0.05; ANOVA followed by Duncan's t- test).*

Females

The individual and the mean serum TSH levels of female patients are given in Tables 8, 9 and Fig 6. The presurgical mean serum TSH concentrations in females were lower than the control values (0.47 ± 0.07 vs 1.51 ± 0.47) but the difference was not statistically significant. As in the male patients, TSH levels remained unchanged postoperatively in all of them.

DISCUSSION

In the present study, we have attempted to assess the hormonal contribution of the pituitary adenomas diagnosed as nonfunctioning, by measuring pituitary hormones in serum before and after surgical removal of the tumour, and in the excised

Table 4: *Serum FSH levels in female patients with NFPA and in age-matched control subjects.*

Patients with NFPA					Control subjects		
	Patient ID	Age (y)	FSH (mIU/ml) Presurgical	Postsurgical	Patient ID	Age (y)	Serum Control FSH (mIU/ml)
Females							
	PA4	35	45.1	5.7	C17	35	6.3
	PA16	42	23.6	7.2	C18	42	8.9
	PA19	35	40.3	34.5	C19	35	10.0

Table 5: *Mean ± SEM serum FSH levels in female patients with NFPA and normal subjects.*

Group	Serum FSH (mIU/ml)
Females	
Control Subjects (n= 03)	8.4± 1.0
Patients with pituitary adenoma (03)	
Presurgical	36.4 ± 6.5*
Postsurgical	15.8 ± 9.3

*Significantly different from postsurgical and control values (P< 0.05; ANOVA followed by Duncan's t- test).

adenoma tissue. Previous literature indicates that NFPAs are morphologically heterogeneous and can be separated into two main categories based on immunohistochemical and electron microscopic appearances[7]. According to this classification one category of NFP-As 'null cell adenomas', includes tumours lacking characteristics of normal adenohypophyseal cells and possessing neither morphological nor immunohistochemical markers indicating their cyto-

Table 6: *Serum TSH levels and TSH content of adenoma tissue, in male patients with NFPA and in age-matched control subjects.*

Patients with NFPA						Control subjects		
	Patient ID	Age (y)	Serum TSH (µIU/ml) Presurgical	Postsurgical	TSH Adenoma tissue (µIU/g)	Patient ID	Age (y)	Serum TSH (µIU/ml)
Males								
	PA1	30	4.5	4.8	67.8	C1	30	0.3
	PA2	35	1.5	0.8	ND	C2	35	0.3
	PA7	22	0.0	0.4	34.4	C3	22	0.2
	PA8	40	2.1	0.7	125	C4	40	6.0
	PA9	55	0.5	0.7	217	C5	55	0.5
	PA10	45	0.3	0.2	227	C6	45	0.3
	PA12	35	0.1	0.2	477	C7	35	0.1
	PA14	50	0.1	0.4	694	C8	50	5.8
	PA18	50	1.9	0.1	176	C9	50	0.3
	PA21	38	0.0	0.4	ND	C10	38	1.3
	PA22	50	0.8	0.1	ND	C11	50	2.6
	PA24	50	0.9	0.6	ND	C12	50	0.6
	PA26	45	1.2	0.6	31	C13	45	0.3
	PA28	42	0.4	0.1	122	C14	42	0.6
	PA29	35	1.2	0.6	599	C15	35	2.6
	PA30	45	0.4	0.1	ND	C16	45	1.6

ND: Non-detectable levels

genesis or direction of differentiation. The second group generally termed as 'silent adenomas', includes tumours exhibiting immunohistochemical and ultrastructural features of some of the recognizable adenohypophyseal cells but without any sign of hormone secretion. These 'silent' adenomas may possess adenohypophyseal cell types characteristic of normal adenohypophyseal tissue.[17] These adenomas have also been shown to express the messenger ribonucleic acid of the related hormone, indicating gene expression and synthesize one or more adenohypophyseal hormones as evidenced by immunocytochemistry.[17-19] In a study of patients with pituitary adenomas in which there was no evidence of excess hormone secretion in presurgical endocrine evaluation,[22] the α- and β-subunits of glycoprotein pituitary hormones were detectable in 68% of the cases.[22] Similar results have been obtained in another study where expression of one or more of the anterior pituitary hormone genes, was found in 12 of the 14 patients with NFPAs, using oligo- nucleotide probes encoding the α-and β-subunits of LH, FSH and TSH.[5]

Table 7: *Mean ± SEM serum TSH levels and TSH content of adenoma tissue, in male patients with NFPA and normal subjects.*

Group	Serum TSH (μIU/ml)	Adenoma tissue TSH content (mIU/g)
Males		
Control Subjects (n = 16)	1.5 ± 0.4	
Patients with pituitary adenoma (n = 16)		175.0 ± 55.8
Presurgical	1.0 ± 0.2	
Postsurgical	0.7 ± 0.2	
Pooled pituitary tissue		198.4

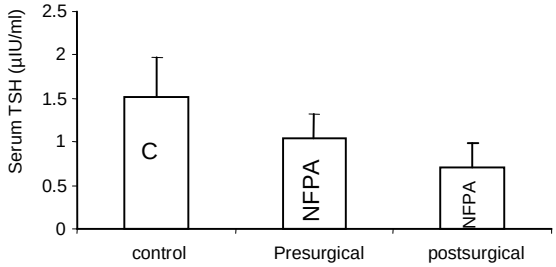

Fig. 5: *Mean ± SEM serum concentration of TSH (μIU/ml) in male patients with NFPA and age-matched control subjects.*

Table 8: *Serum TSH levels in female patients with NFPA and in age matched control subjects.*

Patients with NFPA					Control subjects		
	Patient ID	Age (y)	Serum TSH (μIU/ml) Presurgical	Postsurgical	Patient ID	Age (y)	Serum TSH (μIU/ml)
Females							
	PA4	35	0.5	0.1	C17	35	0.1
	PA16	42	0.3	0.2	C18	42	0.1
	PA19	35	0.4	0.5	C19	35	0.6

Our results indicate that the production and release of intact glycoprotein hormones such as FSH, occurs only in a small proportion of patients with NFPAs. In the present study a hypersecretion of FSH was evident in 4 male and 3 female patients (37%) with NFPAs and in 6 of these patients (4 males and 2 female patients) increased FSH levels reverted to normal serum concentrations indicating that adenoma was not only capable of synthesizing this glycoprotein hormone but also releasing the hormone in peripheral circulation. These observations are further supported by the finding that the FSH content of the adenoma tissue of these patients was significantly higher than those of the rest of the patients with NFPAs and of the control pituitary tissue. Daneshdoost et al[29] in their study found a higher proportion of patients (10 of 38) with NFPAs who had a supranormal

serum concentration of intact FSH and LH, 6 men had supranormal α-subunit concentrations and 6 men had supranormal LH β concentrations. Other studies have reported a low incidence (4-17%) of elevated gonadotropins in patients with NFPAs and these results are generally consistent with our findings.[4,7-11]

Table 9: *Mean ± SEM serum TSH levels in female patients with NFPA and normal subjects.*

Group	Serum TSH (μIU/ml)
Females	
Control Subjects (n = 03)	0.2 ± 0.1
Patients with pituitary adenoma (03)	
Presurgical	0.4 ± 0.07
Postsurgical	0.2 ± 0.1

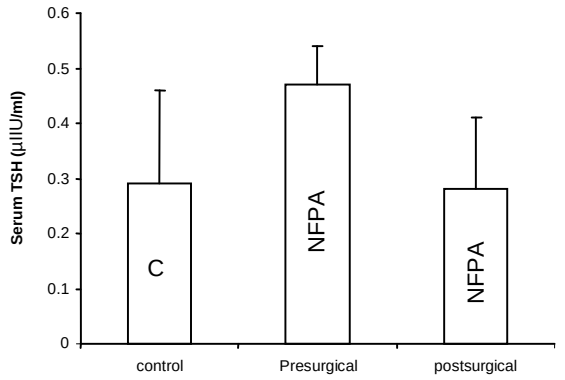

Fig. 6: *Mean ± SEM serum concentration of TSH (μIU/ml) in female patients with NFPA and age-matched control subjects.*

In our study, the presurgical mean serum TSH concentrations in patients with pituitary adenoma were mostly in the lower range and were not significantly different from the control. No significant differences were observed in TSH levels before and following removal of the adenoma. The TSH content of adenoma tissue was either non-detectable or in the low range as compared to the TSH content of the control pituitary tissue. These results indicate that in cases included in this study the synthesis of TSH by the adenoma tissue was negligible. Other studies have also reported low or normal TSH levels in patients with NFPAs and are generally consistent with our findings.[13,14]

The question why most of the NFPAs are clinically or biochemically silent, has not been resolved.[3-5] Some authors have insisted that silent adenomas may produce biologically inactive hormones, precursor proteins, or hormone fragments.[5,7,11] Others have suggested that the abnormalities may occur in exocytosis of hormone from the cell membrane so that despite normal biosynthesis, active secretion of the hormone does not take place.[17,18] Yamada et al[20] and Klibanski[21] are of the opinion that the amount of hormones discharged by the adenoma cells is lower than the normal range to produce any clinical findings, as only a few cells in the tumour are involved in hormone secretion.

It is **concluded** that a significant proportion of NFPAs may actively synthesize and secrete pituitary hormones such as FSH, in amounts that may not be sufficient to produce any overt clinical signs of hormone hypersecretion. The study also indicates the need of monitoring of hormone profile of patients with NFPAs before and after surgery, on an individual basis, for efficient case management.

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CORRIGENDUM

Mona Aziz Ahmad, Rabia Ahmad et al

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The name of the first author may be

read as Mona Aziz and not Mona Aziz Ahmad