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ORIGINAL ARTICLE

The value of prolactin in inferior petrosal sinus sampling with desmopressin stimulation in Cushing's disease

Xiaona Qiao · Hongying Ye · Xiaolong Zhang · Weiwei Zhao · Shuo Zhang · Bin Lu · Xuanchun Wang · Zhaoyun Zhang · Xi Wu · Min He · Xiaolong Zhao · Shiqi Li · Linuo Zhou · Yehong Yang · Renming Hu · Yiming Li

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Abstract Prolactin may reduce false-negative results in diagnosing Cushing's disease (CD) during inferior petrosal sinus sampling (IPSS). Prolactin normalization could improve the accuracy of IPSS in predicting adenoma lateralization in CD. However, none of the previous studies had involved the use of desmopressin during IPSS. Our objective was to examine the utility of prolactin measurement during IPSS with desmopressin stimulation. We conducted a retrospective analysis of 40 patients (including 31 females) with ACTH-dependent Cushing's syndrome who underwent IPSS between 2010 and 2013. Thirty-eight CD patients were partitioned into true positive ($n = 35$) and false negative ($n = 3$). The proportion of improper IPSS venous sampling defined by corresponding IPS:P (inferior petrosal sinus to peripheral) prolactin ratio <1.8 was significantly different between two groups ($P = 0.004$). Applying a prolactin-normalized ACTH IPS:P ratio >0.8 cutoff could increase the sensitivity of IPSS to 38/38 (100 %). Among the 31 patients with histopathologically proven adenoma localization, correct prediction of adenoma lateralization was obtained in 14/31 (45 %) patients by a peak intersinus ACTH gradient of ≥1.4 in baseline and was not improved by desmopressin stimulation. Left–right intersinus gradients of unilateral prolactin-adjusted ACTH IPS:P ratios could increase the correct prediction of adenoma lateralization to 20/31 (65 %) in baseline and 24/31 (77 %) ($P = 0.006$) after desmopressin stimulation, respectively. Prolactin is helpful to adjust negative results of IPSS with desmopressin stimulation. It may improve the accuracy in predicting adenoma lateralization in CD as well.

Keywords Cushing's disease · Desmopressin · Inferior petrosal sinus sampling · Prolactin

Abbreviations

ACTH	Adrenocorticotropin
CD	Cushing's disease
EAS	Ectopic ACTH syndrome
IPSS	Inferior petrosal sinus sampling
UFC	Urinary free cortisol
IPS	Inferior petrosal sinus
IPS:P	Inferior petrosal sinus to peripheral
CRH	Corticotropin-releasing hormone
CV	Coefficient of variation
PRL	Prolactin
IPS:IPS	Inferior petrosal sinus to inferior petrosal sinus

Introduction

Adrenocorticotropin (ACTH)-dependent Cushing's syndrome can be caused by ACTH-dependent Cushing's

Yehong Yang and Linuo Zhou contributed equally to this work.

X. Qiao · H. Ye · W. Zhao · S. Zhang · B. Lu · X. Wang · Z. Zhang · X. Wu · M. He · X. Zhao · L. Zhou (✉) · Y. Yang (✉) · R. Hu · Y. Li
Department of Endocrinology & Metabolism, Huashan Hospital, Fudan University, 12 Middle Urumqi Road, Shanghai 200040, China
e-mail: linuozhouhsh@163.com

Y. Yang
e-mail: yehongyang@fudan.edu.cn

X. Zhang
Department of Radiology, Huashan Hospital, Fudan University, Shanghai 200040, China

S. Li
Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai 200040, China

disease (CD) in 80–85 % cases, by ectopic ACTH syndrome (EAS) in 10–20 % cases, and very rarely by a corticotropin-releasing hormone (CRH)-secreting tumor (ectopic CRH syndrome) [1]. The differential diagnosis of ACTH-dependent Cushing's syndrome continues to be an important challenge in clinical endocrinology.

Inferior petrosal sinus sampling (IPSS) is currently considered the gold standard test to determine the etiology of ACTH-dependent Cushing's syndrome. A petrosal sinus to peripheral ACTH gradient of at least 2.0 in baseline or at least 3.0 after CRH administration is supposed to be diagnostic of CD. The intersinus ACTH gradient of $\geq$1.4 before or after CRH stimulation is suggested to predict the localization, where the maximum gradient value at the various time points is selected for calculation [2]. Desmopressin (a long-acting synthetic vasopressin analogue) can also be used as a stimulator during IPSS, resulting in similar accuracy as CRH with a false-negative rate of 1–10 % [3–6]. Moreover, CRH is not as easily available as desmopressin for clinical use in China, and probably not in some other countries as well [7]. However, neither CRH nor desmopressin stimulations seem to predict the lateralization of adenomas precisely, with an accuracy ranging from 50 to 100 % [3, 8, 9]. The possible reasons may be the variability of venous drainage, catheter misplacement, and improper venous sampling [10].

Previous studies have suggested that the measurement of other anterior pituitary hormones including prolactin during the IPSS procedure may be helpful. Prolactin could be used as an indicator of the pituitary venous effluent to contribute to assist in confirming correct catheter placement and reduce the false-negative results in diagnosing CD [11–13]. One study indicated that prolactin measurement could improve the accuracy of IPSS in predicting adenoma lateralization in CD [14]. However, reports on the use of desmopressin during IPSS are limited, and none has evaluated the significance of prolactin measurement during IPSS with desmopressin administration.

Our aim in this study was dual: first, to examine the utility of prolactin as a marker of proper venous sampling to enhance the accuracy of IPSS to diagnose CD with the desmopressin stimulation; second, to investigate the value of prolactin measurement during IPSS with the desmopressin stimulation for the correct localization of adenoma in patients with CD.

Subjects and methods

Subjects

The study included 40 patients with ACTH-dependent Cushing's syndrome who underwent IPSS at Huashan Hospital between 2010 and 2013 and was approved by the Ethics committee of Huashan Hospital of Fudan University.

Diagnosis of Cushing's syndrome

Cushing's syndrome was diagnosed by typical clinical features, namely increased 24 h urinary free cortisol (UFC) levels (mean of three samples), loss of circadian rhythm in plasma cortisol (at 0800 and 2300 h), and failure of 1 mg dexamethasone overnight to suppress cortisol secretion (normal values <1.8 μg/dl) [15–17]. ACTH-dependent Cushing's syndrome was diagnosed based on an unsuppressed ACTH level [16]. The following criteria were used to confirm the diagnosis of CD: (i) remission of hypercortisolaemia following transsphenoidal surgery or radiosurgery or the development of adrenal insufficiency postoperatively or (ii) demonstration of adenomatous tissue immunoreactive for ACTH on pathological examination [11, 18].

Imaging evaluation

Magnetic resonance (MR)-imaging with and without gadolinium contrast enhancement and standard spin echo sequences was used to identify a pituitary tumor.

IPSS procedure

IPSS was performed by an experienced interventional radiologist. The bilateral femoral veins were cannulated. French sheaths were inserted into both femoral veins and guided catheters were introduced and advanced into the jugular veins bilaterally, then into the inferior petrosal sinus (IPS) at their distal portions. Via the guiding catheters, microcatheters were introduced and selectively placed into the IPS bilaterally and into the cavernous sinuses. Venous angiography and fluoroscopy were used to guide the placement of catheters during IPSS (Fig. 1). Successful catheter placement was defined by demonstration of retrograde flow of contrast into the cavernous sinuses. Blood was slowly withdrawn from both catheters simultaneously and from a peripheral vein. Desmopressin (Hainan Zhonghe Pharmaceutical Co., Ltd) in a dose of 10 μg was then infused into a peripheral vein, and samples were simultaneously obtained from both IPS and peripheral vein 5 and 10 min after the administration of desmopressin.

ACTH, prolactin assays

Plasma ACTH was measured in an automated chemiluminescence immunoassay (Siemens Healthcare Diagnostics, Los Angeles, CA). The intraassay coefficient of variation (CV) was 3.1–9.6 % and the interassay CV was

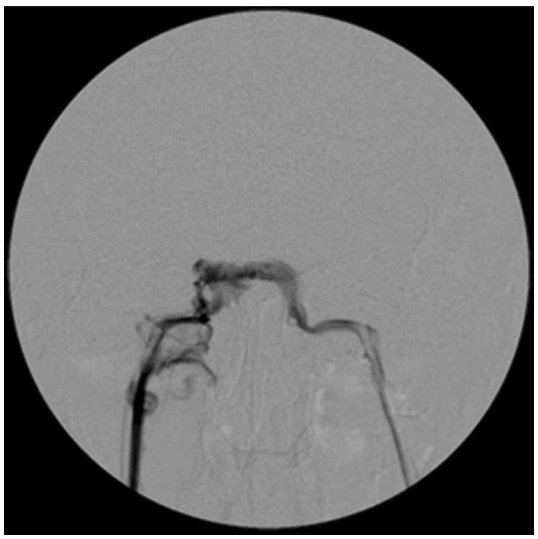

Fig. 1 The standard of successful catheterization. The contrast outlined the anatomy of the inferior petrosal, cavernous, and intercavernous sinuses. Optimal catheter positioning is demonstrated by ipsilateral opacification of the inferior petrosal sinus with contralateral reflux

5.1–9.4 %. Plasma prolactin was measured using an chemiluminescence immunoassay (Siemens Healthcare Diagnostic Inc, Los Angeles, CA), intraassay CV of 4.84–5.02 %, and interassay CV of 3.98–5.07 %.

IPSS analysis

Diagnosis of CD

ACTH ratios of inferior petrosal sinus to the peripheral venous blood (IPS:P) were calculated at each time point, and the peak ratio of at least 2.0 in baseline or at least 3.0 after desmopressin administration was considered diagnostic of CD. In addition, the IPS:P prolactin ratios were calculated at each time point to confirm proper or improper IPS venous sampling, as defined by an IPS:P prolactin ratio of $\geq$1.8 and <1.8, respectively [12]. The dominant ACTH IPS:P ratio was defined as the highest ACTH IPS:P ratio at 0, 5, or 10 min during IPSS. The dominant ACTH IPS:P ratio divided by the concurrent and ipsilateral IPS:P prolactin (PRL) ratios was calculated, which was considered the PRL-normalized ACTH IPS:P ratio. A PRL-normalized ACTH IPS:P ratio >0.8 designated the patients with CD, whereas the ratio <0.6 indicates EAS [12].

Assessment of adenoma lateralization

The gradient of ACTH between the petrosal sinuses (the greatest of the right to left or left to right ratio termed intersinus gradient) was calculated, which was considered the standard ACTH IPS:IPS (inferior petrosal sinus to inferior petrosal sinus) ratio. A peak intersinus gradient of standard ACTH IPS:IPS ratio $\geq$1.4 was used as a criterion for pituitary adenoma lateralization. Additionally, The PRL-adjusted ACTH IPS:IPS ratio was calculated by dividing the IPS ACTH level by a concomitantly sampled IPS prolactin level at each time point. The dominant (highest) intersinus gradient of PRL-adjusted ACTH IPS:IPS ratio was used for estimating the adenoma lateralization, as described in Mulligan's study [14]. The resulting estimates of lateralization by two methods were compared with surgical results.

Statistical methods

Data are given as mean $\pm$ standard deviation. Fisher's exact test was used to analyze the proportion of improper IPS venous sampling. McNemar's test was used to compare test performance between the "standard ACTH IPS:IPS ratio" and the "PRL-adjusted ACTH IPS:IPS ratio" after desmopressin stimulation. Comparisons between means were drawn using Student's t test as data were normally distributed. Analyses were performed using SPSS 17.0(IBM Corporation; Somers, New York). The level of significance was set at 0.05 in all statistical tests.

Results

Clinical characteristics of study subjects

Of the 40 patients, two patients were highly suspected EAS but refused to undergo surgical exploration so that the final diagnosis remained unknown, and the data were excluded in the analysis. Thirty-eight out of 40 patients were diagnosed with CD and were the subject of our study (Table 1). The etiological source of ACTH production was confirmed by definite immunostaining positive for ACTH ($n = 31$), or in case of postsurgical remission ($n = 2$) in spite of the absence of immunological confirmation for ACTH, or remission after pituitary radiosurgery ($n = 5$) [3]. The remission was defined as period of hypocortisolism (adrenal insufficiency) as the normal corticotrophs are suppressed by prior hypercortisolism [19].

The value of prolactin in diagnosing CD

By using a baseline cutoff of $\geq$2 for the peak ACTH IPS:P gradient or $\geq$3 after desmopressin administration, we could diagnose the pituitary source of ACTH in 35 of 38 (92.1 %) patients. The remaining 3 patients in whom the peak ACTH IPS:P gradient was not observed were

Table 1 Clinical and biochemical characteristics of CD patients

Characteristics	Results
Mean age (years)	33.4 ± 12.4
Gender M/F	7/31
0800 h plasma cortisol (μg/dl)	32.1 ± 10.2
24 h UFC (μg/24 h)	780.7 ± 593.7
Plasma ACTH (pg/ml)	91.8 ± 50.5
Mean follow-up (months)	23.4 ± 10.0

CD Cushing's disease, *ACTH* adrenocorticotropin, *M* male, *F* female; 0800 h plasma Cortisol reference range, 6.2–19.4 μg/dl; *UFC* urinary free Cortisol (normal range 30.15–129.13 μg/24 h); plasma ACTH reference range, 0–46 pg/ml

Table 2 Comparison of the rate of improper IPS venous sampling in true-positive results with false-negative results in IPSS

IPSS results	IPS:P PRL >1.8	IPS:P PRL <1.8	*P* value
True positive	31	4	0.004
False negative	0	3	

IPS inferior petrosal sinus, *IPS:P* inferior petrosal sinus to peripheral; *PRL* prolactin; true positive, the patients confirmed with Cushing's disease (CD) and had peak ACTH IPS:P gradient ≥2 in baseline or ≥3 after desmopressin administration; false negative, the patients were confirmed with CD, but did not get peak ACTH IPS:P gradient ≥2 at baseline and ≥3 after desmopressin administration. Improper IPS venous sampling was defined by IPS:P prolactin ratio <1.8

pathologically proved to have CD and got postsurgical remission. The proportion of improper IPS venous sampling defined by corresponding IPS:P prolactin ratio <1.8 between true-positive and false-negative group was significantly different ($P = 0.004$, Table 2). Of the 35 patients whose IPSS results showed true positive, only 4 patients had corresponding IPS:P prolactin ratio <1.8. However, all the 3 patients whose IPSS results showed false negative had corresponding IPS:P prolactin ratio <1.8. One patient had all but one of the IPS:P prolactin ratios less than 1.8 (Table 3); the remaining 2 patients had all of the IPS:P prolactin ratios no more than 1.3.

When the criterion of PRL-normalized ACTH IPS:P ratio >0.8 was used, the number of correct diagnosed patients could reach 38/38 (100 %). All the 3 patients who demonstrated absent IPS:P ACTH gradients (<2 before or <3 after desmopressin administration) showed the PRL-normalized ACTH IPS:P ratio >0.8 (no less than 1.2) and were proved to have CD by pathology (Fig. 2). Regarding the 3 patients with histologically confirmed corticotroph adenoma who failed to reach diagnostic IPS:P gradients, the decision for transsphenoidal surgery was based on 90 % cortisol suppression during high-dose dexamethasone suppression test and the evidence of a pituitary adenoma on MRI imaging.

Lateralization of the adenoma in CD

Identified adenomas were characterized intraoperatively based on the location of the epicenter of the adenoma (on the midline or to one side of the midline). A tumor was considered right or left sided if its epicenter at surgery was off the midline; it was considered midline if its epicenter was directly on the midline. Larger adenomas that occupied both sides of the pituitary gland were considered bilateral. If multiple tissue specimens were resected, their locations were recorded and each specimen was individually assessed for an ACTH+adenoma. The findings of ACTH IPS:IPS ratio, PRL-adjusted ACTH IPS:IPS ratio, MRI, and surgical pathology are outlined in Table 4.

Using a peak intersinus ACTH gradient of ≥1.4 during basal sampling without being normalized by prolactin, we could diagnose the adenoma lateralization correctly in 14/31 (45 %) patients, and the diagnostic accuracy was not improved by desmopressin stimulation. The PRL-adjusted ACTH IPS:IPS ratio during basal sampling could increase the correct prediction of adenoma lateralization to 20/31(65 %). When PRL-adjusted ACTH IPS:IPS ratio after desmopressin stimulation was used, the correct prediction of adenoma lateralization could be observed in 24/31 (77 %) patients ($P = 0.006$, Table 5). Eleven

Table 3 IPSS data of one of the patients with false-negative results

	ACTH (pg/ml)[a]			ACTH ratio		Prolactin (mIU/l)[b]			Prolactin ratio	
Time (min)	P	L	R	L/P	R/P	P	L	R	L/P	R/P
0	64.2	59.8	66.9	0.9	1.0	2,268	1,851	1,321	0.8	0.6
5	59.8	82	78.7	1.4	1.3	1,081	2,247	1,100	**2.1**	1.0
10	82	94.1	127	1.2	1.6	1,611	2,226	1,359	1.4	0.8

The tumor of this patient was located in right pituitary gland

The patient had all but one (*bold*) of the IPS:P prolactin ratios less than 1.8. *IPSS* inferior petrosal sinus sampling, *ACTH* adrenocorticotropin, *P* peripheral, *L* left petrosal sinus, *R* right petrosal sinus, *L/P* left petrosal sinus to peripheral ratio, *R/P* right petrosal sinus to peripheral ratio

[a] ACTH reference range, 0–46 pg/ml

[b] Prolactin reference range, 40.3–530.0 mIU/l

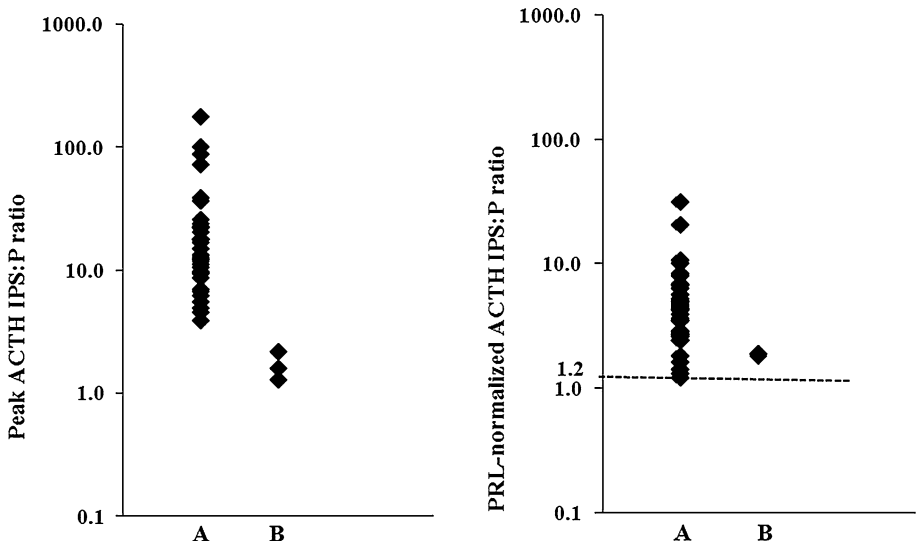

Fig. 2 Peak ACTH IPS:P ratios (*left panel*) and PRL-normalized ACTH IPS:P ratios (*right panel*) in 38 patients with CD. Thirty-five patients with true-positive results of IPSS make up bin *A*, while the three patients with false-negative results are lumped in bin *B*. All patients with false-negative results had a RPL-normalized ACTH IPS:P ratio >0.8, the least one was 1.2 (*dotted line*)

Table 4 The findings of ACTH IPS:IPS ratio, prolactin-adjusted ACTH IPS:IPS ratio, MRI, and surgical pathology in 31 patients with CD who underwent surgery

Variable	Level	*n*	%
Lateralization by maximal IPS:IPS ACTH ratio	Left	12	38.7
	Right	15	48.4
	Midline or bilateral	4	12.9
Lateralization by maximal PRL-adjusted ACTH ratio	Left	17	54.8
	Right	11	35.5
	Midline or Bilateral	3	9.7
MRI findings	Left	14	45.1
	Right	7	22.6
	Midline	5	16.1
	Negative	5	16.1
Surgical pathology	Left	15	48.3
	Right	11	35.5
	Midline	4	12.9
	Bilateral	1	3.2

ACTH adrenocorticotropin, *CD* Cushing's disease, *IPS:IPS* inferior petrosal sinus to inferior petrosal sinus, *PRL* prolactin

Table 5 Comparison of test performance between the standard ACTH IPS:IPS ratio and the PRL-adjusted ACTH IPS:IPS ratio after desmopressin stimulation

ACTH IPS:IPS ratio after desmopressin	PRL-adjusted ACTH IPS:IPS ratio after desmopressin stimulation		
	Correct	Incorrect	*P* value
Correct	13	1	0.006
Incorrect	11	6	

ACTH adrenocorticotropin, *IPS:IPS* inferior petrosal sinus to inferior petrosal sinus, *PRL* prolactin

patients for whom adenoma lateralization failed to be correctly predicted by standard ACTH IPS:IPS ratio after desmopressin stimulation benefited from PRL-adjusted ACTH IPS:IPS-prediction of lateralization. Only one patient with correct adenoma localization by standard ACTH IPS:IPS ratio could not get correct adenoma localization by PRL-adjusted ACTH IPS:IPS ratio after desmopressin stimulation and had a pituitary adenoma in the right side of the pituitary upon histopathological evaluation of the surgical piece (Table 6).

Twenty-six patients out of 38 patients (68 %) had positive MR imaging. Among the 31 patients who underwent surgical exploration and histopathological evaluation, 26 patients had previously displayed MRI findings compatible with a pituitary adenoma, and lateralization was correctly predicted radiologically in 19/31 (61 %) patients. MR imaging was unable to identify 12 patients because the study was either normal ($n = 5$) or erroneously localized the adenoma ($n = 7$) (Table 6). MRI findings were concordant with the localization based on ACTH IPS:IPS ratio and prolactin-adjusted ACTH IPS:IPS ratio in 9/31 patients (29 %) and 14/31 patients (45 %), respectively.

Complications

Except for some minor haematomas of the groin at the site of venous puncture, there have been no other complications in any of these 40 patients.

Discussion

Our data support the contention that prolactin measurement during IPSS with desmopressin stimulation can reduce false-negative results. A cutoff of IPS:P prolactin ratio

Table 6 Comparison of unadjusted and PRL-adjusted ACTH IPS:IPS ratio and MRI findings performance with surgical pathology for adenoma localization in 31 patients with CD who underwent surgery

No	MRI findings	Maximal IPS:IPS ACTH ratio	Lateralization by maximal IPS:IPS ACTH ratio	Maximal PRL-adjusted ACTH IPS:IPS ratio	Lateralization by maximal PRL-adjusted ACTH ratio	Surgical pathology
1	Negative	1.1	M/B	2.2	R	R
2	L	2.4	R	1.5	L	L
3	M	1.5	R	2.2	R	M
4	L	4.2	R	1.9	R	R
5	R	1.1	M/B	1.6	R	R
6	L	2.0	L	1.6	L	L
7	M	9.1	L	5.0	L	L
8	R	5.9	L	4.5	R	R
9	Negative	1.5	L	1.5	L	R
10	L	6.2	L	5.8	L	L
11	L	7.3	R	2.8	L	L
12	L	3.2	R	1.7	R	B
13	**R**	**1.6**	**R**	**1.2**	**M/B**	**R**
14	M	3.0	R	2.0	R	R
15	Negative	3.5	L	4.0	R	R
16	L	15.8	L	7.8	L	L
17	L	19.6	R	5.0	R	L
18	R	6.8	R	1.6	R	R
19	L	2.6	L	3.5	L	L
20	R	12.1	R	4.2	R	R
21	L	3.3	L	2.2	L	L
22	L	1.1	M/B	1.2	M/B	M
23	Negative	1.7	R	1.3	M/B	M
24	R	1.9	L	3.8	L	R
25	M	2.8	R	1.5	L	M
26	L	6.9	R	1.6	L	L
27	Negative	2.8	L	1.9	L	L
28	L	1.5	R	2.0	L	L
29	M	1.3	M/B	1.4	L	L
30	R	1.6	L	8.9	L	L
31	L	16.4	R	3.4	L	L

Only one patient with correct adenoma localization by standard ACTH IPS:IPS ratio could not get correct adenoma localization by PRL-adjusted ACTH IPS:IPS ratio (*bold*) and had a pituitary adenoma in the right side of the pituitary

ACTH adrenocorticotropin, *CD* Cushing's disease; *IPS:IPS* inferior petrosal sinus to inferior petrosal sinus, *PRL* prolactin; *R* right, *L* left, *M/B* midline or bilateral

<1.8 should be used to indicate improper IPS catheterization. Specifically, the presence of an IPS:P prolactin ratio <1.3 associated with a negative ACTH IPS:P ratio should raise the suspicion of an improper IPS venous sampling. Furthermore, PRL-normalized ACTH ratio can enhance accuracy of IPSS to diagnose CD.

Many researchers have attempted to measure some pituitary hormones including GH, TSH, and prolactin during IPSS to reduce the small but meaningful false-negative results in diagnosing CD [11, 20–23]. McNally et al. [21] demonstrated that the ACTH IPS:P ratio adjusted by TRH-stimulated prolactin and TSH got correct lateralization data in 4 of 5 patients with a unilateral pituitary microadenoma. Findling et al. [12] used prolactin as a marker of pituitary venous effluent to help to recognize pituitary venous blood which had not been sampled accurately with CD patients who failed to achieve a diagnostic central to peripheral ACTH gradient during IPSS. An IPS:P prolactin ratio ≥1.8 has been considered a proper IPS venous sampling and a ratio of <1.8 considered an improper IPS venous sampling. But, none of these studies involved the use of desmopressin during IPSS.

Desmopressin, a long-acting synthetic vasopressin analog has been used in the differential diagnosis of ACTH-dependent Cushing's syndrome since 1993, when Malerbi et al. [24] reported exaggerated cortisol secretion in 15 of 16 patients with CD as well as in 2 out of the 15 normal individuals and in one case of ectopic ACTH-producing tumor. This test is based on the overexpression of vasopressin receptors AVPR1B and AVPR2 by a corticotrophic

tumor [25], while subsequent studies described occasional expression of desmopressin receptors in ectopic ACTH secretion [26, 27]. Considering CRH is not available and more costly to afford for patients, desmopressin is a safe and effective alternative to CRH in the setting of IPSS [7].

Limited reports on the use of desmopressin during IPSS showed an accuracy of about 92.1–96 % in differentiating CD and EAS [3, 5], but it did not reliably localize pituitary adenomas. Previous researchers reported the largest series of patients with ACTH-dependent Cushing's syndrome submitted to an IPSS procedure with desmopressin stimulation ($n = 56$) achieving 94.1 % sensitivity in diagnosing CD [5]. Our study used an IPS:P prolactin ratio ≥1.8 to confirm proper IPS venous sampling and <1.8 to confirm improper IPS venous sampling during IPSS with desmopressin administration for the first time. We also had the ACTH IPS:P ratios normalized to the IPS:P prolactin ratios from the corresponding anatomic sites to overcome possible venous drainage or suboptimal catheterization at the same time. We found that all the 3 patients with false-negative IPSS results had corresponding IPS:P prolactin ratio <1.8. The proportion of improper IPS venous sampling between correct results and incorrect results showed a significant difference. Taken together, these results suggest that false-negative findings might be explained by improper placement of the sampling catheter.

The corresponding IPS:P prolactin ratios <1.8 in true positive patients were also observed despite the number was small in our study. Such findings are in line with previous studies' conclusions. For example, Sharma et al. [28] reported that measurement of prolactin levels did not appear to be useful or necessary in patients with an ACTH IPS:P ratio suggestive of CD, because all but one of these patients was properly diagnosed without prolactin measurement in their series. Mulligan's study also concluded that prolactin measurement did not alter data interpretation in positive IPS:P ACTH ratio cases, but was valuable in helping to validate negative results [11].

In our study, all the patients with CD showed the PRL-normalized ACTH IPS:P ratio >0.8, exactly, all ≥1.2. A recent study concluded that PRL-normalized ACTH IPS:P ratio ≥1.3 was indicative of CD and that ≤0.7 was suggestive of EAS but the implication of values between 0.7 and 1.3 remained unclear [13]. Our value of 1.2 was very close to the ratio indicative of CD in Sharma's study [13]. However, the two patients with high suspicion of EAS did not get pathological diagnosis. Therefore, these suggestive results warrant a new study tailored toward the determination of relevant cutoffs for EAS diagnosis.

Findling et al. [12] used the highest ACTH IPS:P ratio at 2, 5, or 10 min after the injection of CRH to define the dominant ACTH ratio, with a potential drawback of misdiagnosis for those patients with a basal ACTH gradient higher than post-CRH. Moreover, they only used pre-CRH prolactin values to calculate the PRL-normalized ACTH IPS:P ratio, which did not account for improper IPS venous sampling after CRH. Therefore, we included ACTH and prolactin values before and after desmopressin stimulation during IPSS to calculate the PRL-normalized ACTH IPS:P ratio. Desmopressin, unlike CRH stimulation during IPSS in other reports [18, 29], seemed not to increase the prolactin level significantly in our study (Fig. 3), which suggests that prolactin might be a more stable indicator of the pituitary venous effluent with desmopressin than CRH stimulation during IPSS.

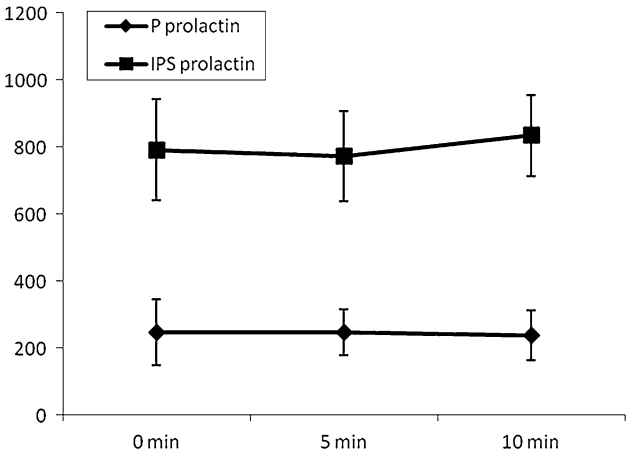

Fig. 3 Mean prolactin levels in the P and IPS ipsilateral to the adenoma. Stimulation with desmopressin did not cause a significant increase in prolactin levels both in the P and IPS samples. *P* peripheral, *IPS* inferior petrosal sinus; prolactin reference range, 40.3–530.0 mIU/l

Our study also supports the hypothesis that prolactin measurement during IPSS with desmopressin stimulation could improve our ability to correctly localize the pituitary adenoma site in CD. Similar with the previously published series [30, 31], our results also displayed a low accuracy rate in predicting adenoma lateralization in only 14/31 (45 %) before desmopressin stimulation, which was not improved by desmopressin stimulation before prolactin normalization. This result made it impossible to direct the surgeon to begin an initial examination of the pituitary gland on the side ipsilateral to the catheter gradient [32]. The addition of prolactin measurements into the test improved our ability to localize pituitary adenoma to 24/31 (77 %) with a significant statistical difference. Seven of our patients did not get a correct prediction by PRL-adjusted ACTH IPS:IPS ratio, which could be explained by pre-existing communication between the cavernous sinuses. This point cannot be modified by prolactin normalization.

There are some limitations to our study. First, it is a retrospective design. Prolactin-adjusted ACTH IPS:IPS

ratios were not used to predict adenoma location prior to surgery or to guide the surgical approach in our patients. Second, there was no patient with EAS so that we could not get the exact value of prolactin in EAS. Third, there was no comparison of CRH and desmopressin in this cohort. Finally, late-night salivary cortisol for the diagnosis of Cushing's syndrome is not carried out in our hospital and was not included in the study [33]. Therefore, further prospective studies with a larger number of patients are expected to confirm these findings. In conclusion, prolactin measurement during IPSS with desmopressin stimulation enhances the accuracy of IPSS to diagnose CD when an absent central to peripheral ACTH gradient is observed. Moreover, it does not alter data interpretation in positive ACTH IPS:P ratio cases. This observation may suggest that false-negative results are most parsimoniously explained by a potential improper sampling. PRL-normalized ACTH IPS:P ratio can then be used to further distinguish between a pituitary and ectopic source of ACTH. Prolactin also may enhance lateralizing capability of IPSS with desmopressin stimulation. However, because of the small but meaningful error prediction rate, thorough exploration of the pituitary gland is still necessary when an adenoma is not discovered based on predicted lateralization by prolactin correction.

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Conflict of interest The authors declared that they have no conflict of interests.

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