Original Article

Bilateral Inferior Petrosal Sinus Sampling in Corticotropin-Dependent Cushing's Syndrome: A Single Center Experience from Western India

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Abstract

Objective: Efficacy of bilateral inferior petrosal sinus sampling (BIPSS) in corticotropin-dependent Cushing's syndrome (CS) for localization and lateralization of excess adrenocorticotropic hormone (ACTH) source, as compared to high-dose dexamethasone suppression test (HDDST) and magnetic resonance imaging (MRI) pituitary, respectively. **Methodology:** Thirteen patients with clinically and biochemically confirmed CS underwent HDDST, MRI pituitary, and BIPSS by an experienced team of intervention neurologist, neurosurgeon, and endocrinologist using percutaneous femoral vein approach. **Results:** Of 13 patients (11 adults and two children) who underwent BIPSS, raised central to peripheral ACTH ratio was achieved in 12 cases, remaining one case being ectopic ACTH secretion (EAS). However, inter IPS gradient >1.4 was achieved in 11 (91.6%) of 12 Cushing's disease (CD) cases before vasopressin stimulation; and in 9 (75%) of 12 CD cases after vasopressin stimulation (*P*-value 0.583). HDDST suppression of more than 50% was present in only ten cases with CD, falsely negating CD in two cases (16.6%), sensitivity 83.3% and specificity 100%. MRI sella demonstrated pituitary microadenoma in 12 cases and macroadenoma in one case. Lateralization by BIPSS and MRI was concordant in 7 (58.3%) out of 12 cases with CD, with rate of remission after transsphenoidal surgery being higher in patients with concordant lateralization by BIPSS and MRI. **Conclusions:** BIPSS is an important investigation to distinguish CD and EAS. BIPSS was superior to HDDST for confirming the source of excess ACTH. Our findings favor the use of BIPSS for localization and pituitary MRI for lateralization of microadenoma.

Keywords: BIPSS-Bilateral inferior petrosal sinus sampling, CD-Cushing's disease, EAS-ectopic ACTH secretion, ectopic ACTH syndrome, HDDST- high-dose dexamethasone suppression test, IPS-Inferior petrosal sinus, LDDST-low-dose dexamethasone suppression test

Introduction

Endogenous Cushing's syndrome (CS) results from the overproduction of cortisol, either due to corticotropin-dependent or corticotropin-independent cause, with a prevalence of 2–3 cases/million/year.[1] Ectopic adrenocorticotropic hormone (ACTH)-secreting tumor may remain occult for a long time, making an accurate diagnosis of corticotropin-dependent CS a challenging issue.

Dynamic gadolinium-enhanced magnetic resonance imaging (MRI) represents the foremost accurate imaging modality for pituitary adenomas. However, MRI has limited power in identifying subcentimeter ACTH-secreting microadenomas because it also identifies 10%–20% of nonfunctioning pituitary incidentalomas.[2-4] Although the presence of a macroadenoma (>10 mm) is very implicative of pituitary disease, this is not true for small lesions which may also be nonsecretory incidentalomas.[2,3] Hence, MRI cannot be completely relied upon to differentiate between Cushing's disease (CD) and ectopic ACTH secretion (EAS). The diagnostic accuracy of the stimulatory and inhibitory

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biochemical hormone tests as described in literature has been 65%–100% sensitivity and 60%–100% specificity for the high-dose dexamethasone suppression test (HDDST) and 70%–93% sensitivity and 95%–100% specificity for the Corticotropin-releasing hormone (CRH) test.[5]

The Consensus Statement on Diagnosis and Complications of CS suggests 6 mm cut off for a pituitary lesion detected by MRI to be considered corticotroph adenoma, and proceeded directly for surgery without IPSS. However, this data was mainly based on the size of pituitary lesions in healthy controls.[4] Ten percent of the general population harbors incidental pituitary tumors[5,6] and pituitary incidentalomas as large as 14 mm have also been seen in patients with EAS.[7-9] The suggested 6 mm cut off provides specificity only up to 96% for accurately diagnosing CD.[8] Hence, bilateral inferior petrosal sinus sampling (BIPSS) is taken into account to substantiate the source of ACTH as pituitary or ectopic, with high sensitivity and specificity. CRH stimulation during BIPSS has been well documented in literature for maximizing the sensitivity of BIPSS.[4] However, due to the poor availability of CRH in our country, desmopressin and vasopressin have also been used as corticotroph stimulant during BIPSS with similar sensitivity.[10] Vasopressin acts on V3 (arginine vasopressin receptor 1B) receptors present on corticotroph cells in the anterior pituitary and has been shown to have ACTH secretagogue effect.[10]

In this study, we have summarized our experience of BIPSS in 13 patients with corticotropin-dependent CS and compared HDDST with BIPSS for localization of the source of excess corticotropin. We also assessed the accuracy of MRI pituitary and BIPSS in lateralizing pituitary adenoma with respect to treatment outcomes.

Methodology

Subjects

Patients admitted in the indoor facility of our department with corticotropin-dependent CS, established from clinical history and examination, basal 8 am and 11 pm serum cortisol, overnight dexamethasone suppression test (ONDST), low and high dose dexamethasone suppression test (LDDST and HDDST, respectively), and ACTH levels were recruited. After biochemical confirmation, patients underwent BIPSS after an interval of approximately 7 days.

BIPSS procedure and interpretation

The BIPSS procedure was performed by the same experienced team of intervention neurologist, neurosurgeon and endocrinologist. Bilateral femoral veins were accessed and catheters were maneuvered into inferior petrosal sinuses under radiological guidance. Blood was simultaneously withdrawn from both petrosal catheters and from ipsilateral peripheral veins at different time points for ACTH measurement and collected in cold test tubes. Basal samples were taken 5 min and 1 min before vasopressin stimulation; then, vasopressin (one unit diluted in 10 mL saline) was injected into a peripheral vein and repeated samples were taken after 5 min and 10 min.[10] Blood samples were instantly cold transported to the laboratory for ACTH assay (using Immulite 1000 Chemiluminescence immunoassay). Central to peripheral ACTH ratio was calculated for right and left side. For the diagnosis of Cushing's disease (CD), basal central to peripheral ACTH ratio was ≥2 and/or post-vasopressin stimulation ratio ≥3 was endorsed. For lateralization to a given side, a ratio of ≥1.4 was used. In case, these ratios were not met, the diagnosis of ectopic CS was considered.

Treatment and outcome

All the patients diagnosed as CD underwent transsphenoidal surgery (TSS) as per lateralization by MRI of sella. BIPSS may not reliably identify the tumor site based on side-to-side gradient of 1.4,[11-13] and hemi-hypophysectomy based on IPSS lateralization cured only 50% of patients in one study, an outcome no better than chance.[14] Suspected tumor tissue and pituitary tissue adjacent to the tumor was resected and pathologically examined. Therapeutic outcome was categorized as "remission" if the serum cortisol level was below 5 μg/dL measured within the first 2 weeks after TSS, and residual if not meeting the said criterion.[15-17]

Results

Baseline characteristics

Data was collected for 13 patients who underwent BIPSS from March 2018 to March 2020. No severe adverse effects occurred in any of the patients during or after the procedure. Among the 13 patients (eleven adults and two children), 12 were female and one child was the only male patient. Mean age of study population was 31.2 ± 11.6 years and median duration of disease was 2.6 years. The average 8:00 am and 11 pm cortisol level was 34.63 ± 15.76 μg/dL and 24.40 ± 16.68 μg/dL, respectively. Morning fasting ACTH level (141.20 ± 135 pg/mL) was elevated in all patients suggestive of ACTH-dependent CS. All but three cases had more than 50% drop in cortisol levels with HDDST. MRI sella demonstrated pituitary microadenoma in 12 cases and macroadenoma in one case. Based on abnormal clinical and chest X-ray findings, contrast-enhanced CT chest was done for two cases (case five and seven in Table 1) that demonstrated a space-occupying lesion in right lung in both the cases. Also, one case (case no 8 in Table 1) had a CT abdomen done before presenting to us. It showed an adrenal stalk lesion, which was, however, not supported by our biochemical findings. Detailed clinical characteristics and Cortisol suppression tests are listed in supplementary Tables 2 and 3 respectively.

Results of BIPSS before and after vasopressin stimulation

Inferior petrosal sinuses could be successfully catheterized in all our patients. Based on ACTH values, 12 patients were diagnosed as CD and one patient (case five in Table 1) as EAS although MRI sella showed 1 × 1 mm microadenoma in this case also. Raised central/peripheral ACTH ratio was achieved in all twelve CD cases, both before and after vasopressin stimulation. Inter IPS gradient >1.4 was achieved in 11 (91.6%)

Table 1: HDDST, lateralization on BIPSS and MRI, and treatment outcome of study cohort

Case No.	Diagnosis	HDDST suppression >50%	IPSS Lateralization	MRI Sella	TSS done on side	Outcome
1.	CD	Yes	Left	4 × 5 mm left microadenoma	Left	Remission
2.	CD	No	Left	4 × 3 mm right microadenoma	Right	Remission
3.	CD	Yes	Right	5 × 3 mm right microadenoma	Right	Remission
4.	CD	Yes	Right	5.8 × 4 mm right microadenoma	Right	Remission
5.	EAS	No	None	1 × 1 mm right microadenoma	Right	Expired
6.	CD	Yes	Left	5 × 3 mm left microadenoma	Left	Residual
7.	CD	Yes	Left	9 × 8 mm left microadenoma	Left	Residual
8.	CD	Yes	Right	6 × 9 × 7 mm left microadenoma	Left	Residual
9.	CD	Yes	Left	3 × 6 × 2 mm left microadenoma	Left	Remission
10.	CD	No	Left	6 × 6 mm right microadenoma	Right	Expired
11.	CD	Yes	Right	3.7 × 2.8 mm left microadenoma	Left	Residual
12.	CD	Yes	Right	28 × 21 × 14 mm left macroadenoma	Left	Medical
13.	CD	Yes	Right	6.1 × 6 mm right microadenoma	Right	Medical

Table 2: Baseline characteristics of study cohort

Parameter	Mean±SD	Range
Age (years)	31.2±11.6	10-50
Disease duration (years)	2.6	0.5-5
Weight (kg)	65.76±12.28	44-86
BMI (kg/m^2)	28.44±5.08	20.83-39.10
SBP (mmHg)	148.3±14.67	112-170
DBP (mmHg)	94.20±9.18	70-106
FBS (mg%)	149±49	96-205
HbA1c (%)	8.53±2.97	5.8-15.3
Basal 8:00 am Cortisol (µg/dL)	34.63±15.76	16.7->75
11 pm Cortisol (µg/dL)	24.40±16.68	8.9->75
ACTH (pg/mL)	141.20±135.7	32.4-470

of 12 CD cases (excluding one case of EAS) before vasopressin stimulation; and in nine (75%) of 12 CD cases after vasopressin stimulation (*P*-value 0.583). Detailed results are available in supplementary Tables 4 and 5.

Sensitivity and specificity of HDDST versus BIPSS for localization of CD

HDDST has previously been the mainstay of biochemical differential diagnosis between the pituitary and ectopic ACTH-dependent syndrome. In our series, HDDST suppression >50% was present in only 10 out of these 12 cases, falsely negating CD in two cases (16.7%). The sensitivity and specificity of HDDST turned out to be 83.3% and 100%, respectively. However, BIPSS could correctly diagnose CD in all 12 cases and was found to be superior to HDDST for differentiating CD and EAS.

Adenoma lateralization and treatment outcomes

In our cohort, MRI sella revealed a pituitary microadenoma in twelve cases, including one case (case five) with EAS and pituitary macroadenoma in one case (case twelve in Table 1). Lateralization by BIPSS and MRI was concordant in seven (58.3%) out of 12 cases with CD. Out of the total 13 patients who underwent BIPSS, two patients (case 5 with EAS and case 10 with CD in Table 1) expired before definitive surgery owing to acute cardiovascular complications. Of the remaining 11 CD patients, two (case 12 and 13 in Table 1) denied surgery and were started on medical management with oral ketoconazole. Nine patients underwent TSS based on MRI localization and were postoperatively confirmed with biopsy of excised tissue. Five of these are in remission while four patients have residual disease. It was observed that 4 out of 5 (80%) patients in remission had concordant lateralization results on MRI sella and BIPSS for corticotroph adenoma, while 50% cases with residual disease had discordant lateralization on IPSS and MRI sella (*P*-value 0.812). Case three and case four were pediatric cases and underwent TSS followed by hypocortisolism and hypopituitarism. Both the children are in remission and under regular follow-up.

Refer to Table 1 for the results of HDDST, lateralization on BIPSS and MRI, and treatment outcome of study cohort.

Discussion

CS is easily diagnosed clinically owing to its characteristic features. However, anatomical localization of the source of excess ACTH is challenging. Definite treatment of CS is surgical removal of ACTH-secreting lesion, a prerequisite for which is distinct localization of ACTH-secreting source. Pituitary MRI localizes microadenoma in only 50%–60% of the cases attributable to the small size of corticotroph adenoma which can lead to falsely negative diagnosis.[6,18]

HDDST >50% suppression has long been used as a criterion to distinguish EAS with CD and holds substantial importance in the management of patients with ACTH-dependent CS when facilities for BIPSS are not readily available. It could correctly predict pituitary adenoma in 10 out of 12 cases with CD in our series, with false-negative result in two (16.6%) cases. HDDST had a sensitivity of 83.3% and specificity of 100% as compared to BIPSS which was 100% sensitive and specific for

Table 3: Cortisol suppression tests and pituitary imaging

Case No.	8:00 am Cortisol (μg/dL)	11:00 pm cortisol (μg/dL)	ONDST (μg/dL)	LDDST (μg/dL)	HDDST (μg/dL)	ACTH (pg/mL)	MRI Sella
1.	33.5	31.07	21.09	23.09	2.95	156	4 × 5 mm left microadenoma
2.	26.6	21.80	20.06	16.91	13.7	53.1	4 × 3 mm right microadenoma
3.	37.08	18.69	25.78	29.32	9.88	109	5 × 3 mm right microadenoma
4.	46.31	22.10	9.25	11.96	14.63	79.6	5.8 × 4 mm right microadenoma
5.	>75	>75	>75	>75	61.26	470	1 × 1 mm right microadenoma
6.	16.7	15.32	12.29	5.40	1.29	102	5 × 3 mm left microadenoma
7.	35.4	8.90	3.56	17.10	7.6	69.7	9 × 8 mm left microadenoma
8.	20.8	16.11	5.36	7.45	5.01	32.4	6 × 9 × 7 mm central and left microadenoma
9.	35.1	24.14	12.50	10.30	14.91	406	3 × 6 × 2 mm left microadenoma
10.	31.14	27.1	29.9	20.84	26.01	97.9	6 × 6 mm microadenoma
11.	29.85	16.24	8.82	13.54	10.58	99	3.7 × 2.8 mm microadenoma
12.	14.42	10.53	4.86	3.63	4.5	65.3	28 × 21 × 14 mm macroadenoma
13.	48.25	30.28	41.94	18.46	8.35	95.9	6.1 × 6 mm microadenoma

Table 4: Adrenocorticotropic hormone values of basal and post vasopressin stimulation from both petrosal sinuses and femoral veins

Central/peripheral ACTH ratio

Case No.	−05 min		−01 min		+05 min		+10 min	
	Right	Left	Right	Left	Right	Left	Right	Left
1.	4.35	11	1.89	11.25	1.69	>14.5	1.5	>12.65
2.	1.94	22.6	9.63	22.9	>31.25	30.52	11.2	11.4
3.	3.99	0.39	4.13	0.35	3.81	0.60	2.9	1.31
4.	9.9	1.13	4.47	4.07	3.0	1.16	6.8	1.54
5.	1.35	1.34	1.06	1.06	1.024	1.01	1.03	0.94
6.	1.92	1.75	1.82	2.11	19.11	19.11	3.14	3.5
7.	9.61	4.8	8.4	7.40	7.96	7.96	1.5	6.9
8.	2.10	1.47	1.97	1.94	15.2	10.5	7.8	6.6
9.	16.8	27.1	20.2	25.4	11.1	15.3	3.6	10.4
10.	5.07	9.33	4.3	3.55	5.14	5.41	4.32	4.60
11.	8.78	0.49	12.81	1.3	9.06	1.99	19.98	1.25
12.	3.67	3.38	3.76	2.47	3.35	2.48	3.14	2.10
13.	3.60	0.27	7.39	1.31	11.46	4.42	6.86	2.19

Inter IPS gradient

Case No.	−05 min	−01 min	+05 min	+10 min
1.	2.54	5.1	>8.56	>8.44
2.	11.6	2.38	0.97	1.01
3.	10.06	11.83	6.26	2.21
4.	8.8	1.12	2.5	4.4
5.	1.0	0.99	1.01	1.09
6.	1.09	1.16	1	1.11
7.	1.97	1.14	1	4.5
8.	1.42	1.01	1.43	1.17
9.	1.60	1.25	1.37	2.82
10.	1.84	1.22	1.05	1.06
11.	17.83	9.68	4.5	6.78
12.	1.08	1.51	1.35	1.49
13.	12.9	5.6	2.59	3.125

distinguishing CD and EAS in our cohort. As evident in literature also, BIPSS is superior to HDDST for confirming CD and should be preferred at centers where the facility and technical expertise is available. Though we did not encounter any procedure-related complication in our cohort, transient nausea, vomiting, ear discomfort, and

Table 5: Highest central to peripheral adrenocorticotropic hormone ratio and interpetrosal adrenocorticotropic hormone gradient

Highest central/peripheral ACTH ratio (basal)	Highest central/peripheral ACTH ratio (poststimulation)	Highest inter IPS ratio (basal)	Highest inter IPS ratio (poststimulation)	Diagnosis*	Lateralization
11	>14.5	5.1	>8.56	CD	Left
22.92	31.25	11.6	1.01	CD	Left
4.13	3.81	11.83	6.26	CD	Right
9.9	6.8	8.8	4.4	CD	Right
1.35	1.03	1.16	1.11	EAS	None
2.11	3.5	1.16	1.11	CD	Left
9.61	7.96	1.97	4.5	CD	Left
2.10	15.2	1.42	1.43	CD	Right
27.1	15.3	1.60	2.82	CD	Left
9.33	5.41	1.84	1.06	CD	Left
12.81	19.98	17.83	6.78	CD	Right
3.76	3.35	1.51	1.49	CD	Right
7.39	11.46	12.9	3.125	CD	Right

*CD-Cushing's disease; EAS- Ectopic ACTH secretion

catheter site hematoma formation have been reported as risks with BIPSS.[10,19]

Numerous studies on BIPSS have used CRH and desmopressin either alone or in combination for stimulating corticotroph cells with satisfactory results.[20,21] However, neither CRH nor intravenous desmopressin was available at our center; hence, we used vasopressin for corticotroph stimulation. Kotwal *et al*. in 2016 for the first time reported a significant increment in ACTH values and satisfying results after vasopressin injection (1.0 unit diluted in 10 mL saline) in six patients.[10] In their series also, despite the increment in ACTH levels, central/peripheral ACTH ratio >2 was achieved in all CD cases even without vasopressin stimulation, similar to our findings. In our cohort, we also found that inter IPS ratio >1.4 was achieved in greater number of patients before rather than after vasopressin stimulation (91.6% vs. 75%, respectively). Thus, vasopressin stimulation with 1 unit dose did not provide any added benefit in localization or lateralization of corticotroph adenoma with BIPSS in our experience. However, further studies in larger cohorts and probably higher vasopressin doses are needed to confirm our findings.

BIPSS series have shown the sensitivity of 88%–100% and specificity of 67%–100% in the localization of the CD.[10,22] Similarly in our study, BIPSS could accurately localize the source of ACTH production in all the cases. The role of BIPSS in lateralization of the lesion in CD has been questioned, with accuracies ranging from 50% to 100%.[15,23,24] In our cohort, lateralization by BIPSS and MRI was concordant in only seven (58.3%) out of 12 cases with CD. Our findings corroborate with the well-accomplished evidence favoring the use of BIPSS for localization of CD and pituitary MRI for more accurate lateralization of corticotroph adenoma.[12,25,26]

Considering the impact of precise localization with MRI or BIPSS on treatment outcomes, four out of five (80%) patients in remission had concordant lateralization on MRI sella and BIPSS, while 50% cases with residual disease had discordance in lateralization (*P*-value 0.812). Our study suggests that treatment outcomes are better when the MRI localization of adenoma is concordant with BIPSS localization, as compared to discordant cases. Though the difference was not statistically significant, it paves the way for further study in larger cohorts before accepting or refuting the observation.

Conclusions

With the ability to confirm the central secretion of ACTH, BIPSS is an important investigation to distinguish CD and EAS. In our cohort, vasopressin stimulation (1 unit in 10 mL saline) during BIPSS did not provide any added benefit in localization or lateralization of corticotroph adenoma. BIPSS was found to be superior to HDDST for localization and confirmation of CD with sensitivity of 100% as compared to HDDST with sensitivity of only 83.3%. MRI is more accurate for lateralization of corticotroph adenoma and forms basis for surgery. Favorable treatment outcomes and remission rate after TSS are higher in patients with concordant lateralization of pituitary adenoma by BIPSS and MRI. Hence, BIPSS may be recommended in all patients with ACTH-dependent CS, provided our findings are validated in larger cohorts as our sample size was small with only thirteen cases.

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Conflicts of interest

There are no conflicts of interest.

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