Original Article

Performance of Vasopressin Stimulated Bilateral Inferior Petrosal Sinus Sampling in Corticotropin Dependent Cushing's Syndrome with Negative or Equivocal 3 Tesla Contrast Enhanced Magnetic Resonance Imaging of Pituitary

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Abstract

Introduction: Corticotropin releasing hormone (CRH)-stimulated bilateral inferior petrosal sinus sampling (BIPSS) is the most accurate procedure in the differential diagnosis of adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome (CS) with a sensitivity of 88–100% and a specificity of 67–100%. However, CRH is not available globally currently. We undertook this study of BIPSS using lysine vasopressin (LVP) as an agent to stimulate the release of ACTH from corticotrophs. Our objective was to assess the accuracy of LVP-stimulated BIPSS in differentiating Cushing's disease (CD) from ectopic ACTH syndrome (EAS) with negative or equivocal 3T contrast-enhanced MRI (CEMRI). **Methods:** Seventeen patients with clinically and biochemically confirmed ACTH-dependent CS with equivocal or negative CEMRI pituitary underwent BIPSS using LVP as a stimulating agent. **Results:** Of seventeen patients who underwent BIPSS, nine patients had a raised central-to-peripheral ACTH ratio and were classified as having CD that was confirmed on histopathology following transsphenoidal sinus surgery. Remaining eight patients, who did not show a raised central-to-peripheral ACTH ratio, were classified to have EAS. All patients with EAS underwent contrast-enhanced computerised tomography of the neck, chest, and abdomen and/or Gallium 68 DOTANOC positron emission tomography/computerised tomography. Seven out of eight patients demonstrated solitary pulmonary nodule in the lung (bronchial carcinoid), and one patient had a mass in the thymus (thymic carcinoid). **Conclusion:** BIPSS using LVP confirmed the source of ACTH excess correctly in all the patients with ACTH-dependent CS without the loss of specificity.

Keywords: BIPSS – bilateral inferior petrosal sinus sampling, CD – Cushing's disease, CS – Cushing's syndrome, EAS – ectopic ACTH secretion, LVP – lysine vasopressin

Introduction

Endogenous Cushing's syndrome (CS) may be caused by excess adrenocorticotropic hormone (ACTH) production (80–85%), usually by a pituitary corticotroph adenoma (CD), less frequently by an extra pituitary tumour (EAS), or very rarely by a tumour secreting CRH (ectopic CRH syndrome).[1] CS can also be ACTH-independent (15–20%) when it results from excess secretion of cortisol by unilateral adrenocortical tumours, either benign or malignant, or by bilateral adrenal hyperplasia or dysplasia.[1-4]

Pituitary is the source of excess ACTH secretion in approximately 80% cases of ACTH-dependent CS.[1-6] Remaining 10–20% cases of ACTH hypersecretion are due to EAS.[1-6] Common tumours associated with EAS are bronchial carcinoid, small-cell lung carcinoma, pancreatic neuroendocrine tumour (PNET), thymic carcinoid, medullary carcinoma of thyroid (MTC),

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and pheochromocytoma.[6-9] For the aetiological diagnosis and localisation of the culprit lesion, a progressive probabilistic stepwise approach should be followed. Biochemical tests like high-dose dexamethasone suppression test (HDDST), plasma ACTH, CRH stimulation test, and desmopressin stimulation test might help in differentiating pituitary from the ectopic source of ACTH hypersecretion.[1-10] Peripheral CRH stimulation test has been described to show a sensitivity and specificity of 70%–93% and 95%–100%, respectively, while HDDST has been described to show a sensitivity and specificity of 65%–100% sensitivity and 60%–100%, respectively.[10]

Pituitary MRI with gadolinium enhancement is the cornerstone of investigations in ACTH-dependent CS given the frequency of CD. In a patient with a classic clinical presentation and dynamic biochemical studies compatible with CD, a cut-off of 6 mm pituitary lesion on MRI has been suggested for diagnosis of corticotroph adenoma.[4] CEMRI reveals a discrete pituitary adenoma in up to 60% of patients with CD. Although it identifies 10%–20% of non-functioning pituitary incidentalomas, the majority of these lesions are less than 5 mm in diameter. Yogi-Morren *et al*.[11] evaluated the accuracy of the 6 mm tumour size cut-off value in the differentiation between patients with CD and EAS and reported a specificity of 96% for the diagnosis of CD.

BIPSS has been established as a gold standard tool in differential diagnosis of ACTH-dependent CS to localise the source of ACTH hypersecretion as pituitary or ectopic with high sensitivity and specificity.[10,11] Ovine or human CRH and/or desmopressin have been used to stimulate corticotropes during BIPSS to improve the accuracy of the procedure with equal potency.[12-14] Recently, there was breakdown in the lyophiliser, leading to interruption of production of CRH by the Ferring pharmaceuticals, resulting in unavailability of CRH worldwide.[15] With the non-availability of CRH, a combination of alternative tests including desmopressin test, HDDST, pituitary MRI, and whole-body thin-slices CT have been recommended. In cases of discordance in the results of the pituitary source of ACTH secretion, clinical features suggestive of ectopic origin of ACTH excess (e.g., severe hypertension, oedema, sarcopenia, sudden onset of clinical picture, hypokalaemia, severe hypercortisolism), or equivocal CEMRI pituitary, desmopressin-stimulated BIPSS is recommended.[16] However, IV desmopressin is also not available in India.

Animal studies have shown that vasopressin is a more potent stimulus for ACTH secretion than CRH by acting on V1b (V3) receptor expressed on anterior pituitary gland.[17,18] LVP is an anti-diuretic hormone found in pigs. It is similar to arginine vasopressin (AVP), which is found in humans, except that LVP has lysine in place of arginine as the eighth amino acid. LVP has been used to stimulate corticotroph cells during BIPSS with similar sensitivity in some previous studies.[18-20] However, these studies included very few patients with EAS. It is hypothesised that more potent stimuli of LVP could raise the number of false-positive responses in patients with EAS, which may compromise the specificity of the procedure. Therefore, in this study, we sought to determine whether the application of the strong stimulation with LVP affects the specificity of the procedure in differential diagnosis of ACTH-dependent CS.

In this study, we have summarised our experience of LVP-stimulated BIPSS done at our centre in 17 patients with ACTH-dependent CS with negative or equivocal 3T CEMRI pituitary including eight consecutive patients with histologically confirmed EAS.

Materials and Methods

Patient inclusion

The study was conducted at the Department of Endocrinology of our institute from January 2018 to October 2022. A total of 38 patients were diagnosed as CS based on clinical history, physical examination, 8 am serum cortisol, 8 am plasma ACTH level, overnight dexamethasone suppression test (ONDST), and low-dose dexamethasone suppression test (LDDST). Serum cortisol ≥1.8 μg/dL was considered non-suppressible after ONDST and LDDST.[5] Midnight serum cortisol was measured to establish abnormal circadian rhythm of cortisol, and a value >1.8 μg/dL was considered abnormal.[5] A value of plasma ACTH >15 pg/mL was taken as a cut-off to define ACTH dependence.[5] Thirty-one patients out of 38 were diagnosed to have ACTH-dependent Cushing's syndrome, and the remaining seven patients were diagnosed as ACTH-independent CS. All seven patients who had ACTH-independent CS were excluded from the study.

All 31 patients with histopathologically proven ACTH-dependent CS (8 patients with EAS and 23 patients with CD) underwent dynamic CEMRI of the pituitary gland (3 Tesla Magnetom Vida, Siemens Heathineers, Erlangen, Germany). Out of 31 patients with ACTH-dependent CS, 3 patients had pituitary macroadenoma (>10 mm), while 11 patients had well-defined microadenoma with >6 mm size having discrete margins on CEMRI of pituitary. These 14 patients were excluded from the final analysis. Five patients had equivocal CEMRI pituitary, and 12 patients had negative CEMRI pituitary. Equivocal MRI pituitary was defined as having hypo enhancing focal lesion in pituitary of <6 mm in size and/or without discrete margins.[21] LVP-stimulated simultaneous BIPSS was performed in all the 17 cases of ACTH-dependent CS in whom pituitary imaging was negative or equivocal [Figure 1].

BIPSS procedure

On the day of BIPSS procedure, 8 am plasma cortisol was elevated (>10 μg/dL) in all the patients, thus excluding the possibility of cyclical CS.[22] The BIPSS procedure was performed by a single interventional neurologist at our hospital. BIPSS was done after catheterising both inferior petrosal sinuses using a percutaneous bilateral femoral vein approach. A criss-cross technique was used to negotiate inferior petrosal sinus. This crossed catheterisation method allowed us to negotiate more difficult left internal jugular vein from the closer right femoral sheath. The catheter was positioned

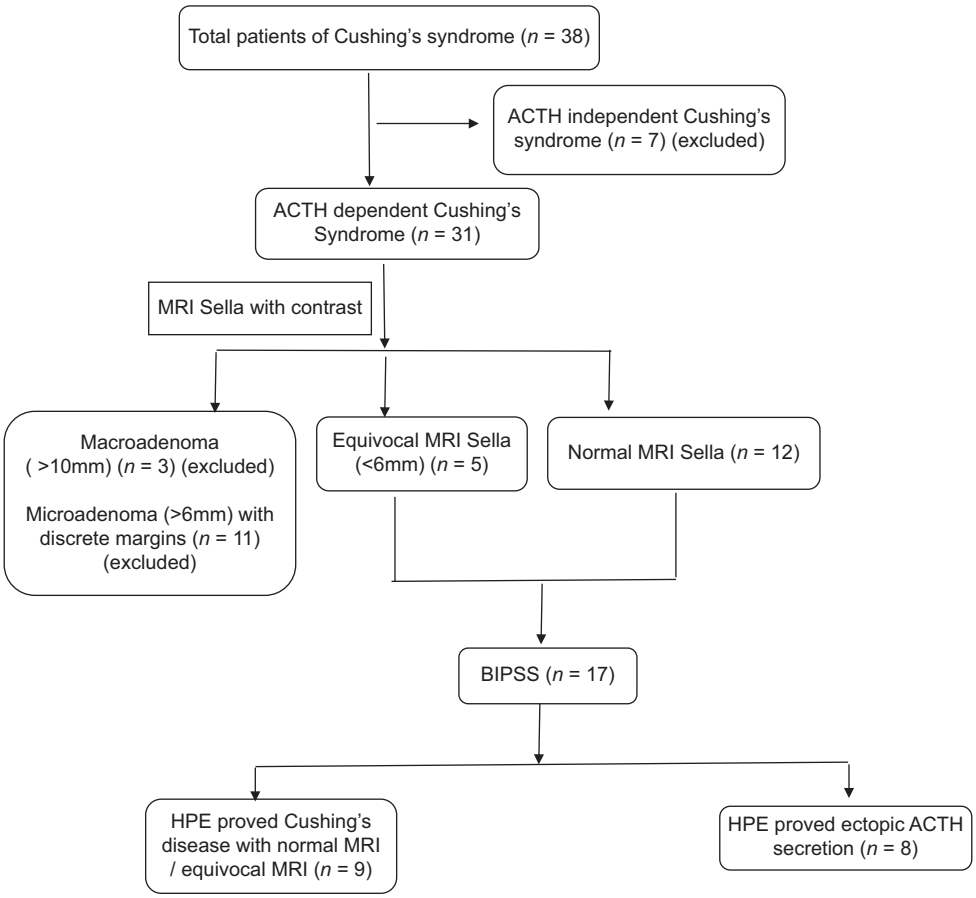

Figure 1: Overview of the study. MRI – Magnetic Resonance Imaging, BIPSS – Bilateral Inferior Petrosal Sinus Sampling, HPE – Histopathological Examination, ACTH – Adrenocorticotrophic Hormone

in the inferior petrosal sinus at the junction of the vertical and horizontal segments. The position was confirmed by venogram using a small amount of non-ionic iodinated contrast material before sampling in all patients. Catheterisation of the bilateral inferior petrosal sinuses was successful in all the patients.

Prior to stimulation, blood was slowly withdrawn over 2 minutes from both catheters simultaneously and from ipsilateral peripheral vein for plasma ACTH and serum prolactin measurement. Subsequently, 5 units of LVP (Vpress by Neon pharmaceuticals) was diluted in 10 ml saline and infused into a peripheral vein slowly over 5 minutes. Samples for plasma ACTH were simultaneously collected over 2 minutes from both inferior petrosal sinuses and peripheral veins at 3 min, 5 min, 10 min, and 15 min after the administration of LVP in cold test tubes.

Samples for plasma ACTH measurements were kept in ice until the completion of the procedure. Later on, they were spun at 4 degrees C, and the plasma was separated and stored at -20 degrees C until analysed. Patients were closely monitored during and 6 hours after the procedure for any adverse effect.

BIPSS analysis

Localisation

Ratios of plasma ACTH values from bilateral inferior petrosal sinuses and peripheral vein were calculated (IPS:P) at each time point. BIPSS was considered diagnostic for a pituitary source of ACTH hypersecretion if the basal ratio (before LVP administration) was ≥2 or if the highest ratio after LVP administration was ≥3. In cases where these ratios were not met, the diagnosis of EAS was considered. The central: peripheral prolactin ratio was >1.8 in all cases considered to have EAS on BIPSS.[23]

Adenoma lateralisation

The inter-petrosal gradient ratios were calculated between the two petrosal sinus samples at each available time point. This ratio was considered predictive of lateralization if it was ≥1.4.

Surgery and histopathology

Patients with a confirmed peripheral source of ACTH hypersecretion based on BIPSS underwent CECT neck, chest, and abdomen and/or Ga^{68} DOTANOC PET/CT on a case-by-case basis. Six out of eight patients with EAS underwent resection of the respective tumour. Patients with a pituitary source of ACTH hypersecretion underwent TSS. The final diagnosis of EAS and CD was made on histopathology.

Disease remission and disease persistence

Remission was defined as post-operative serum cortisol (8 am) of <5 µg/dL at the end of the first week, and disease persistence

was defined as post-operative serum cortisol (8 am) >5 µg/dL at the end of the first week.[21,22]

Assay

Serum cortisol and ACTH were measured by electro-chemiluminescence-immuno-assay (ECLIA) (ELECSYS, Roche Cobas Diagnostics). The inter- and intra-assay coefficients of variation (CV) for plasma ACTH were 4.9% and 8.3%, respectively, while for serum cortisol, they were 6.2% and 7.2%, respectively.

Statistical analysis

Statistical package for the social science (SPSS), version 23 has been used for statistical analysis. All categorical variables were expressed in actual numbers/percentages, and the continuous variables were expressed in mean ± standard deviation. The Student independent t-test was performed to compare basal cortisol, ACTH, ONDST, and LDDST between CD and EAS groups. Sensitivity and positive predictive values were derived for various tests in this study by generating a classical 2 × 2 contingency cross table. For calculating the concordance for localising and lateralising the ACTH source, simple manual case-by-case comparison was done.

Ethical aspects

This retrospective study was approved by the ethical committee of our institution (IEC, Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan), vide letter number 2023/1736 dated 24 November 2023. Written informed consent was obtained from all patients in the study and use of the patient data for research and educational purposes. The procedures in the study follow the guidelines laid down in the Declaration of Helsinki 1964.

Results

Baseline characteristics

Data were collected from 17 patients who underwent BIPSS from January 2018 to October 2022. Among the 17 patients, 11 were males and six were females. One out of 17 patients was a child who was 15 years of age. The study population had a mean age of 34.8 ± 12.9 years. The mean 8 am serum cortisol and plasma ACTH were 32.2 ± 20.7 µg/dL and 142.1 ± 100.5 pg/mL, respectively. Mean ONDST cortisol and LDDST cortisol were 22.3 ± 9.9 µg/dL and 21.0 ± 11.6 µg/dL. Mean plasma ACTH was significantly higher in EAS patients as compared to CD patients ($P = 0.045$). Mean basal cortisol, ONDST cortisol, and LDDST cortisol values were found to be statistically insignificant between the two groups [Table 1]. MRI of the pituitary was normal in 12 patients, while it was equivocal in five cases. Detailed description of biochemical tests performed is shown in Table 1.

Table 1: Biochemical tests

	Cushing's Disease	Ectopic ACTH secretion	*P*
Mean basal cortisol	29.26±11.03 SD	35.52±28.67 SD	0.552
Mean ACTH	91.30±18.5 SD	199.31±125.1 SD	0.045
Mean ONDST	19.25±7.02 SD	25.88±11.99 SD	0.166
Mean LDDST	18.98±13.16 SD	23.2±9.93 SD	0.433

ACTH – Adrenocorticotrophic Hormone, ONDST – Overnight Dexamethasone Suppression Test, LDDST – Low Dose Dexamethasone Suppression Test, SD – Standard Deviation

Results of BIPSS before and after LVP stimulation

All nine patients with histologically proven CD had pre- and post-LVP stimulation central/peripheral ACTH ratios ≥2 and ≥3, respectively, with sensitivity and positive predictive value (PPV) of 100%. All eight patients with histologically proven EAS had pre- and post-LVP stimulation central/peripheral ACTH ratios <2 and <3, respectively, with specificity of 100% and a negative predictive value of 100%. Two out of 8 patients showed very high LVP-stimulated plasma ACTH compared to unstimulated plasma ACTH from the peripheral vein during BIPSS procedure with a negative central: peripheral gradient (IPS:P <3). A pre- and post-LVP-stimulated inter-IPS gradient of ≥1.4 was achieved in eight cases (88.8%) out of nine CD cases [Tables 2 and 3].

Sensitivity and specificity of HDDST versus BIPSS for localisation of CD

In our series, HDDST suppression >50% was present in two-third (6/9) patients with CD, while HDDST suppression <50% was present in three-fourth (6/8) patients with EAS. The sensitivity, specificity, PPV, and NPV of HDDST were 66.6%, 80%, 81.8%, and 72.8%, respectively. However, BIPSS could correctly diagnose CD in all nine cases and EAS in all eight patients. It was found to be superior to HDDST in differentiating CD from EAS [Tables 2 and 3].

Adenoma lateralisation and treatment outcomes

MRI of the pituitary was negative in 12 patients, and it was equivocal in five cases. All nine patients with a pituitary source of ACTH hypersecretion underwent TSS of corticotroph adenoma. Concordance in lateralising adenoma between BIPSS and TSS was observed in 6/9 patients (66%). Six patients achieved remission after surgery, while three patients had persistent disease. One patient (patient no. 8) out of the three patients with persistent disease died because of post-operative complications.

After BIPSS showed the peripheral source of ACTH hypersecretion in eight patients, CECT neck chest and abdomen and/or Ga^{68} DOTANOC PET/CT was done to find the source of ectopic ACTH hypersecretion. Seven out of eight patients demonstrated a suspicious lesion in the lung, and one patient had a mass in thymus. All these eight patients underwent image-guided biopsy. Seven patients were diagnosed to have bronchial carcinoid, and the remaining one was diagnosed to have thymic carcinoid with positive ACTH staining. Six out of seven patients underwent segmental resection or lobectomy for bronchial carcinoid. The patient with thymic carcinoid was found to be unresectable, so he was treated with bilateral adrenalectomy. One patient (patient no. 14) went into remission after surgery but died due to post-operative complications. One

Table 2: Clinical and biochemical characteristics of patients with ACTH-dependent CS

Case no.	Age	Sex	Basal Cortisol	Basal ACTH	ONDST Cortisol	LDDST Cortisol	HDDST Cortisol	Final diagnosis	Treatment	Outcome
1	34	Female	21.6	63.9	16.9	12.2	9.4	CD	TSS	Remission
2	30	Male	16.0	86.0	8.0	4.6	7.8	CD	TSS	Remission
3	15	Male	40.0	91.3	27.1	49.3	36.0	CD	TSS	Remission
4	41	Female	21.5	86.1	14.0	6.2	9.3	CD	TSS	Persistent disease
5	23	Male	24.0	78.0	14.8	25.0	19.6	CD	TSS	Remission
6	35	Female	28.0	87.9	17.9	16.8	9.4	CD	TSS	Remission
7	37	Female	52.0	121.6	32.1	12.9	16.8	CD	TSS	Remission
8	35	Male	33.0	87.0	21.6	25.0	22.1	CD	TSS	Persistent disease, Died, Post-operative complications
9	72	Female	27.3	120.0	18.66	15.43	9.38	CD	TSS	Persistent disease
10	39	Male	24.9	127.0	43.8	28.2	29.0	EAS	Bilateral Adrenalectomy	Remission, Chemotherapy
11	46	Male	27.0	174.0	21.9	19.2	15.8	EAS	SPN excision	Remission
12	30	Male	29.0	139.0	21.1	23.7	19.4	EAS	SPN excision	Remission
13	30	Male	25.9	253.0	21.9	23.0	27.0	EAS	SPN excision	Remission
14	18	Male	24.8	102.0	19.7	43.0	32.2	EAS	SPN excision	Remission → Died due to Post-operative complications
15	45	Female	28.6	110.5	18.6	14.1	12.4	EAS	SPN excision	Remission
16	25	Male	18.0	482.0	14.2	10.1	16.0	EAS	SPN excision	Remission
17	38	Male	106.0	207.0	45.9	25.0	41.2	EAS	-	Died, Sepsis with MODS

ACTH – Adrenocorticotrophic Hormone, ONDST – Overnight Dexamethasone Suppression Test, LDDST – Low Dose Dexamethasone Suppression Test, CD – Cushing's disease, EAS – Ectopic ACTH Secretion, MODS – Multi Organ Dysfunction Syndrome, SPN – Solitary Pulmonary Nodule, TSS – Transsphenoidal Surgery

Table 3: Inferior Petrosal Sinus/Peripheral ACTH ratios and localisation of lesion on BIPSS, CEMRI, and TSS

Case No.	LVP	Basal IPS/P ACTH ratio	Maximum stimulated IPS/P ACTH ratio	Basal Inter sinus ACTH ratio	Maximum Inter sinus ACTH ratio	BIPSS localisation (stimulated)	CEMRI localisation	TSS localisation
1	5 units	12.32	31.50	4.49	10.08	Right	Right	Right
2	5 units	6.78	14.3	6.41	2.65	Right	Right	Right
3	5 units	10	10.83	5.17	10.86	Right	Normal	Left
4	5 units	9.8	58	1.32	25.5	Right	Left	Left
5	5 units	9.56	15.2	1.6	1.66	Left	Left	Left
6	5 units	9.31	10.5	4.35	7.23	Right	Normal	Right
7	5 units	1.83	4.75	1.22	21.4	Left	Normal	Left
8	5 units	2.92	8.24	1.33	1.17	Not lateralised	Normal	Left
9	5 units	4.15	14.81	2.16	10.52	Right	Left	Right
10	5 units	1.07	1.11	1.09	1.21	-	Normal	Ectopic
11	5 units	1.35	1,47	1.09	1.26	-	Normal	Ectopic
12	5 units	0.56	1.16	1.03	1.24	-	Normal	Ectopic
13	5 units	1.04	1.45	1.07	1.77	-	Normal	Ectopic
14	5 units	0.93	0.95	1.10	1.03	-	Normal	Ectopic
15	5 units	1.16	1.38	1.05	1.02	-	Normal	Ectopic
16	5 units	0.89	1.16	1.06	1.08	-	Normal	Ectopic
17	5 units	1.12	1.31	1.18	1.01	-	Normal	Ectopic

ACTH – Adrenocorticotrophic Hormone, BIPSS – BSilateral Inferior Petrosal Sinus Sampling, TSS – Transsphenoidal Surgery, CEMRI – Contrast Enhanced Magnetic Resonance Imaging, IPS - Inferior Petrosal Sinus, P – Peripheral LVP – lysine vasopressin

patient (patient no. 17) with bronchial carcinoid died due to sepsis and multi-organ dysfunction (MODS) before definitive surgery. Patient no. 10, who underwent bilateral adrenalectomy, is currently receiving chemotherapy for metastatic thymic carcinoid. Remaining five patients are in remission till the last follow-up [Tables 2 and 3].

Side effects of LVP used in BIPSS for stimulation and procedure-related complications

A transient increase in blood pressure was the most common side effect associated with the use of LVP. It was present in 6/17 (35.2%) patients who underwent the procedure. 4/17 (23.5%) patients developed transient bradycardia during BIPSS. One

patient developed severe bradycardia (heart rate <40 beats per minute) and was treated with IV atropine with successful reversal. Five patients had transient nausea and abdominal discomfort. There were no procedure-related complications in any of the patients.

Discussion

Traditionally, HDDST has been used in the differential diagnosis of ACTH-dependent CS. The sensitivity and specificity of the 2-day HDDST have been reported to range from 65 to 100% and from 60 to 100%, respectively.[10,24] In our study, the sensitivity, specificity, PPV, and NPV of HDDST were 66.6%, 80%, 81.8%, and 72.8%, respectively. This is similar to the study by Flack *et al*.,[25] who evaluated 118 patients with surgically confirmed cases of CD. The study reported the sensitivity and specificity of HDDST to be 78% and 83%, respectively. Another study by Yan Yang *et al.* from China reported the sensitivity, specificity, and PPV of HDDST to be 70.3%, 77.8%, and 94.7%, respectively.[26] The major problem with HDDST is its diagnostic inaccuracy. Some patients with severe CD, especially those with large pituitary tumours and high cortisol levels, may show a negative response, while nearly 10% of patients with EAS show a positive response.[26]

On the contrary, CRH-stimulated BIPSS has a high sensitivity and specificity of 88–100% and 67–100%, respectively. CRH-stimulated BIPSS is therefore considered as the most accurate test in the differential diagnosis of ACTH-dependent CS. Various studies in the past have also used LVP as the stimulating agent during the BIPSS procedure. Therefore, this study was performed to evaluate the diagnostic accuracy of LVP as a stimulating agent for the ACTH release during BIPSS to localise and lateralise the source of ACTH in patients with CS with negative or equivocal 3T CEMRI of pituitary. This study showed that LVP confirmed the source of ACTH excess correctly in all the patients of CS. LVP-stimulated BIPSS correctly lateralised the pituitary adenoma in two-third of the patients with CD.

Previous studies have demonstrated significantly higher peak ACTH levels from dominant IPS samples in patients with CD who received stimulation with LVP during BIPSS, compared with those who received stimulation with CRH.[18,27] Such a high stimulus may provide some diagnostic advantage over the traditional CRH stimulation by reducing the proportion of patients with false-negative BIPSS due to poor stimulation of corticotropinoma by CRH. However, there is a possibility that a stronger stimulation may affect the specificity of the procedure. This study was conducted to study this aspect of LVP usage in BIPSS stimulation.

In the present study, the strong stimulation with LVP was administered to all consecutive histologically confirmed cases with EAS that were diagnosed in our department. None of the eight patients with EAS tested had a false-positive outcome during the study. Therefore, these data demonstrate that the application of a stronger stimulation by vasopressin during BIPSS in EAS patients does not compromise the specificity of the procedure. This issue is particularly critical for an inadequately suppressed pituitary in cases with periodic ectopic hypercortisolaemia where a more potent stimulus may possibly lead to a higher rate of false-positive gradients. None of the eight EAS displayed an IPS/P ratio of ≥3 when these patients were tested during a hypercortisolaemic phase in our study. Nevertheless, further studies with a greater number of patients with EAS are still required to obtain more information with regard to the specificity of such a strong stimulation during BIPSS. Interestingly, this lack of an IPS/P gradient ≥3 occurred despite a significant ACTH rise during the procedure in two out of eight patients. As previously shown, this rise in peripheral ACTH originates from the ectopic source and is due to the stimulation of the ectopic ACTH-secreting tumour by LVP mediated via V2 receptor and V3 receptor.[27,28] This explains the lack of positive IPS/P ACTH ratio in these two patients with stimulated peripheral ACTH.

BIPSS is a relatively safe procedure in the experienced hands, but neurological side effects such as medial medullary syndrome, pontine haemorrhage, subarachnoid haemorrhage, groin haematoma, or internal jugular venous (IJV) thrombosis have been described.[29] Additional side effects seen with the use of LVP are hypertension, bradycardia, nausea, headache, and abdominal pain. In our study, 1/17 patient developed transient bradycardia during IPSS with a heart rate of 40 per minute and treated with atropine, following which his vitals remained stable throughout the procedure. There have been case reports of severe bradycardia with the use of 5–10 units of intra-myometrial administration of LVP during uterine myomectomy.[30] Rest of the patients had minor side effects that were transient in nature and did not require specific treatment.

In our study, incidence of EAS is high compared to CD. This could be because of the referral bias. The limitations of our study were retrospective nature and a small number of patients. The strength of our study lies in having the highest number of confirmed cases of EAS, compared to previous studies using vasopressin in ACTH-dependent CS.

Conclusion

BIPSS using LVP confirmed the source of ACTH excess correctly in all the patients with ACTH dependent CS without the loss of specificity. In conclusion, based on the data presented in this study, we suggest that even stronger stimulation with LVP is associated with a high sensitivity (100%) but no loss of specificity.

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Authors contribution

The concept of the study was given by Dr. Rajeev Kasliwal, Dr. Gaurav Goyal and Dr. S.K. Sharma. The designing was

done was Dr. Puneet Shivnani and Dr. Jyoti Sharma. Data of the study was acquired by Dr. B.S Sharma, Dr Pankaj Gupta, Dr. Dr. Rajeev Kasliwal, and Dr. Puneet Shivnani. The data was analysed by Dr. Rajeev Kasliwal, Dr. S.K. Sharma, Dr. Vineet Mishra, Dr Dinesh Yadav and Dr Gaurav Goyal. Manuscript was prepared and edited by Dr. Puneet Shivnani, Dr. Rajeev Kasliwal, Dr. Jyoti Sharma and Dr. S.K. Sharma. Statistical analysis was done by Dr. Akash Mishra and Dr Sandeep Garg. Definition of intellectual content was given by Dr BS Sharma, Dr. Pankaj Gupta and Dr. Vineet Mishra. Manuscript review guarantor were Dr. Rajeev Kasliwal and Dr S.K. Sharma.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

In accordance with the journal's guidelines, we are committed to transparency and reproducibility in research. The data supporting the results of this study will be made available by the corresponding author upon reasonable request.

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