Original Article

Internal jugular vein adrenocorticotropic hormone estimation for diagnosis of adrenocorticotropic hormone-dependent Cushing's syndrome: Ultrasound-guided direct jugular vein sample collection

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ABSTRACT

Aim of Study: To assess the utility of internal jugular vein (IJV) / peripheral adrenocorticotropic hormone (ACTH) gradient in determining the etiology of ACTH- dependent Cushing's syndrome. **Materials and Methods:** Patients with ACTH-dependent Cushing's syndrome, (except children less than 12 years), had IJV blood collection under ultrasound guidance using a linear 7 MHZ probe. Blood was collected with a 21 G needle at the level of mandible with the patient in supine position. Six ml of blood was collected sequentially from right and left internal jugular veins for ACTH and prolactin estimation. Peripheral blood for ACTH and prolactin was taken from a previously placed IV cannula in the antecubital vein. **Results:** Thirty patients (20 F, 10 M, age 14 to 50 yrs) were enrolled for this study. Source of ACTH excess was pituitary in 22, ectopic ACTH in 4, and unknown in 4 cases. Using an IJV: Peripheral ACTH ratio of ≥ 1.6, 15 out of 22 Cushing's disease patients were correctly identified. However, 1 out of 4 ectopic Cushing also had IJV: Peripheral ratio ≥ 1.6. Overall, it had sensitivity of 68% with specificity of 75% while MRI pituitary and HDDST had sensitivity of 86% and 59%, respectively, with specificity of 100% each. **Conclusion:** IJV: Peripheral ACTH gradient was observed in 68% of patients with Cushing's disease. Simultaneous IJV and peripheral sample collection with CRH stimulation may improve sensitivity and specificity of this test.

Key words: Adrenocorticotropic hormone estimation, Cushing's disease, Cushing's syndrome, internal jugular vein sampling

Introduction

Pituitary corticotroph adenomas are responsible for the majority (80%) of cases of Adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome. These are usually small tumors and are not always detected on MRI.[1-2] ACTH producing tumors elsewhere in the body account for the other 20% cases. These ectopic ACTH producing tumors may also be very small / remain occult for long number of years. Therefore, identifying and localizing the source of excess / abnormal ACTH secretion is the most critical step in the management of ACTH-dependent Cushing's syndrome.

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Bilateral inferior petrosal sinuses sampling is widely used as part of diagnostic evaluation of patients with ACTH-dependent Cushing's syndrome.[3] Presence of a significant ACTH gradient between venous samples collected from inferior petrosal sinus and peripheral vein suggests a pituitary source. Other sites like the cavernous sinus[4] and internal jugular vein[5] have also been used for central ACTH estimation. Although central to peripheral ACTH gradient is observed in the basal state, sensitivity of the test is further improved with samples obtained after CRH stimulation.[5-7] Super selective venous catheterization through femoral vein (Seldinger technique) is used for collecting ACTH sample.[3-5] This is an invasive procedure requiring infrastructure and skilled manpower, which is not always available, especially in resource-poor countries. Here, we report results of a

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pilot study using USG-guided direct internal jugular vein puncture for sample collection to assess central {internal jugular vein (IJV)}/ peripheral ACTH gradient in patients with ACTH-dependent Cushing's Syndrome.

Materials and Methods

Patients

Patients with ACTH-dependent Cushing's syndrome were the subjects for this study. Inclusion criteria were clinical and biochemical evidence of hypercortisolism and plasma ACTH level greater than 10 pg/ml. Children (age less than 12 years) were excluded. Each patient underwent high dose dexamethasone-suppression test and gadolinium-enhanced MRI of pituitary with post-contrast dynamic sequences. Patients with normal pituitary or lesion < 5 mm on MRI underwent additional tests like contrast-enhanced CT scan of neck, chest, abdomen, and DOTA-TOC PET CT and or FDG PET CT scan. Patients who had no detectable source for ACTH and the very ill patients with pituitary ACTH-dependent Cushing's disease underwent bilateral adrenalectomy. MRI for pituitary was done 6 months later and if normal, repeat examination was advised 1 year later.

Internal jugular vein blood collection was done under ultrasound guidance in the radiology department by a dedicated radiologist. Linear probe (7 MHZ) was used to localize IJV in neck. Blood was collected with a 21 G needle at the level of mandible with the patient in supine position. Needle was inserted keeping tip towards medial wall of IJV. Six ml of blood was collected sequentially from right and left internal jugular veins for ACTH and prolactin estimation. Peripheral blood for ACTH and prolactin was taken from a previously placed IV cannula in the antecubital vein. The whole procedure was completed within 8 to 10 minutes. Blood was collected in pre-chilled plastic tubes containing ethylene diamine tetra acetic acid and was sent to laboratory immediately. After cold centrifugation, the plasma was kept frozen at -20^0 C until assay. Prolactin was used as a marker of pituitary effluent.[8]

ACTH estimation was done with electrochemiluminescence immunoassay (ECLISA) using two monoclonal antibodies specific for ACTH (9-12) and for the C-terminal region (ACTH 36-39).[9] The measuring range of this assay is 1-2000 pg/ml with inter and intra assay CV < 6%. Ratios of central-to-peripheral concentration were calculated for ACTH.

Final diagnosis of Cushing's disease was made when pituitary corticotroph adenoma was confirmed on histopathological examination or remission of hypercortisolism following pituitary surgery or appearance of pituitary adenoma (MRI) following bilateral adrenalectomy. Diagnosis of ectopic ACTH excess was made on identification of an ACTH producing tumor elsewhere in the body. Those where an ACTH source could not be identified were grouped under 'ACTH source unknown.'

This being a pilot study, a sample of 30 was planned based on the number of subjects likely to be available over a 2-year period. The study protocol was approved by the institutional ethics committee.

Results

This study was carried out over 24-month period starting from June 2007 at a tertiary care center. Thirty patients (20 females and 10 males) were enrolled for this study. Age ranged from 14 to 50 years (29 ± 10). High dose dexamethasone suppression test and MRI for pituitary were done for all patients. Sixteen patients had CECT chest and abdomen, 7 each had DOTA-TOC PET and FDG PET as additional investigations. Ultrasound-guided internal jugular vein sample collection was done for ACTH measurement along with peripheral venous sample for all 30 patients. All patients tolerated the procedure well. Other than mild discomfort in the neck (10 patients), there were no adverse events following the procedure.

Source of ACTH excess was pituitary in 22 (micro adenoma 19, macro adenoma 3) and ectopic ACTH in 4 (from thymic carcinoid in 2 and from bronchial carcinoid in 2). Source of ACTH excess could not be localized in 4 cases.

MRI showed lesions in 19 of the 22 patients with Cushing's disease while none of the patients with ectopic ACTH excess had any pituitary lesion. Thirteen of these 22 with Cushing's disease had more than 50% suppression with high dose dexamethasone suppression test while none of the patients with ectopic ACTH excess showed suppression [Table 1].

Central / peripheral ACTH ratio ranged from 0.28 to 10. It was more than 3 in 6, > 2 in 12 and ≥ 1.6 in 20 patients. Out of the 20 with central peripheral ratio ≥ 1.6, 15 had pituitary corticotroph tumor (13 ipsilateral, 2 contralateral) and 1 had a thymic carcinoid. ACTH source could not be identified in 4. Among the 10 with central peripheral gradient less than 1.6, 7 had pituitary corticotroph adenoma and 3 were ectopic.

Discussion

The differentiation of Cushing's disease from ectopic source of ACTH hyper secretion is sometimes difficult because both the tumors may give similar responses to conventional tests like high dose dexamethasone suppression, Metyrapone test etc. Contrast-enhanced dynamic MRI can detect micro adenomas in 60% to 80% of

Table 1: Results of magnetic resonance imaging, high dose dexamethasone suppression test, and Internal jugular vein sampling

Test	Pituitary n = 22	Ectopic n = 4	Unknown* n = 4	Sensitivity	Specificity	Positive predictive value	Negative predictive value
MRI	19	0	1	86	100	100	57
HDDST	13	0	2	59	100	100	30
IJV: P ratio≥1.6	15	1	4	68	75	94	30

*For calculation of sensitivity and specificity, unknown was excluded

cases of Cushing's disease.[10-13] Sometimes, small adenomas detected on MRI may not be the lesion responsible for ACTH hyper secretion. Prevalence of microadenomas in healthy population varies from 10% to 27%.[2,14] Therefore, selective venous sampling was developed to confirm hyper secretion of ACTH from the pituitary gland.

P C Scriba reported the first successful ACTH estimation in samples obtained from internal jugular vein (IJV) in patients with ACTH-dependent Cushing's syndrome in 1966.[15] He had demonstrated IJV / peripheral vein ratio 1.5 ± 0.15 in 4 out of 5 Cushing's disease patients. There was absence of C: P gradient in 3 ectopic Cushing's patients. After that, there are several reports of successful IJV sampling.[16-19] The first case series of 10 patients (6 Cushing's disease and 4 ectopic) showed that IJVS was non-diagnostic in detecting pituitary lesions.[18] Drury *et al*[19] showed that IJVS had detected 9 out of 10 pituitary-dependent Cushing's syndrome. However, there were 13 patients in his study (out of 23) who had either undiagnosed source or ectopic ACTH-dependent Cushing's syndrome. As better assays for ACTH and non-invasive imaging techniques for diagnosis of pituitary tumor became available, this procedure became less popular.[20]

Oldfield *et al*[6] reported their experience with petrosal sinus sampling with and without CRH for the differential diagnosis of Cushing's syndrome. An IPSS: P ratio of ≥ 2 in the basal samples identified 205 of 215 patients with Cushing's disease (sensitivity 95% and specificity 100%); a ratio of ≥ 3 after CRH stimulation identified all 203 patients with Cushing's disease (100% specificity and sensitivity). However, there were 32 patients with unconfirmed diagnosis in this group of 278 who had undergone IPSS. Subsequent studies have shown that this test is also not free from false positive and false negative results.[7,21-23] Some of the false negatives were explained on the basis of variations in the venous anatomy.[21,24,25]

Erickson *et al*[26] compared IJVS with IPSS in 35 patients with ACTH-dependent Cushing's syndrome (32 CD and 3 ectopic). Using the basal central / peripheral ACTH ratio of > 2, IJVS diagnosed 15 (47%) of the 32 patients with Cushing's disease while 29 (91%) could be diagnosed with IPSS. When a lower cut off (1.6) was used, the sensitivity increased to 86%. Ilias *et al*[27] also compared IJVS with IPSS in patients with ACTH-dependent Cushing's syndrome. ACTH values from IJVs were lower compared to IPSS, possibly due to venous dilution. Basal central / peripheral ACTH ratio showed considerable overlap between patient groups but with CRH stimulation, sensitivity increased to 83% at 100% specificity. There were 4 patients with abnormal petrosal sinus anatomy and negative IPSS results in this study. JVS correctly identified pituitary source for ACTH hyper secretion in all these 4 patients.

Both IPSS and IJVS require expertise that is often not available at many medical centers and although rare, some serious complications like intracranial hematoma, brain stem infarct, and thromboembolism[28] have been reported with IPSS.

We used USG-guided direct venipuncture (IJV) for sample collection for ACTH estimation. Peripheral blood was collected from antecubital vein through previously placed catheters. Thirty patients, 20 females and 10 males, age ranging from 14 to 50 years were enrolled in this study. Twenty-two had Cushing's disease while 4 had ectopic source for ACTH hypersecretion. For 4 cases, source of ACTH could not be documented. The IJV: Peripheral ACTH ratio ranged from 0.28 to 10. Out of 20 patients with IJV: P ratio ≥ 1.6, 15 were pituitary and 1 ectopic ACTH excess (thymic carcinoid). There were 4 cases where ACTH source was not identified. These included 3 patients with normal MRI, and 1 had a 5 mm lesion on MRI. Three of the 4 patients with ectopic ACTH hyper secretion had IJV peripheral ratio less than 1.6.

The 4 cases where ACTH source was not been identified were excluded to calculate sensitivity and specificity of the IJV: Peripheral ACTH ratio. This study showed sensitivity of 68% and specificity of 75% in detecting Cushing's disease with cut off ≥ 1.6. These results are comparable to some of the earlier studies[18] where central peripheral ratios were calculated without CRH stimulation. We have a final diagnosis in only 26 of the 30 cases. The other 4 cases will require further follow up to identify source of ACTH excess. Some of these may be Cushing's disease while others may have occult ectopic or as yet unknown / non- tumorous cause for hypercortisolism.[29-31] Several large series of patients with Cushing's syndrome, no definite diagnosis is available in 10% to 15% of patients even after 10 to 20 years of follow-up.[6,22,32]

The main limitation of this study is the number of patients with unknown ACTH source. The IJV samples were collected sequentially from the right and left IJVs, and one peripheral sample was collected during this time for the first 28 patients. Only for last 2 patients was simultaneous peripheral sample collected with each IJV sample. Simultaneous IJV and peripheral vein sample collection with CRH stimulation may improve sensitivity and specificity of this test.

References

1. Newell-price J, Bretagne X, Grossman AB, Nieman LK. Cushing's syndrome. Lancet 2006;367:1605-17.
2. Hall WA, Luciano MG, Doppman JL, Patronas NJ, Oldfield EH. Pituitary magnetic resonance imaging in normal human volunteers: Occult adenomas in the general population. Ann Intern Med 1994;120:817-20.
3. Jehle S, Walsh JE, Freda PE, Post KD. Selective use of bilateral inferior petrosal sinus sampling in patients with ACTH-dependent Cushing's syndrome prior to transsphenoidal surgery. J Clin Endocrinol Metab 2008;93:4624-32.
4. Teramoto A, Nemoto S, Takakura K, Sasaki Y, Machida T. Selective Venous sampling directly from cavernous sinus in Cushing's syndrome. J Clin Endocrinol Metab 1993;76:637-42.
5. Doppman JL, Oldfield EH, Nieman LK. Bilateral sampling of the internal jugular vein to distinguish between mechanisms of ACTH- dependent Cushing's syndrome. Ann Intern Med 1998;128:33-6.
6. Oldfield EH, Doppman JL, Nieman LK, Chrousos GP, Miller DL, Katz DA, *et al*. Petrosal sinus sampling with and without CRH for the differential diagnosis of Cushing's syndrome. N Engl J Med 1991;325:897-905.
7. Colao A, Faggiano A, Pivonello R, Giraldi FP, Cavagnini F, Lombardi G. Inferior petrosal sinus sampling in the differential diagnosis of Cushing's syndrome: Results of an Italian multicenter study. Eur J Endocrinol 2001;144:499-507.
8. Findling JW, Kehoe ME, Raff H. Identification of patients with Cushing's disease with negative pituitary ACTH gradients during inferior petrosal sinus sampling: Prolactin as an index of pituitary venous effluent. J Clin Endocrinol Metab 2004;89:6005-9.
9. Talbot JA, Kane JW, White A. Analytical and clinical aspects of ACTH determination. Ann Clin Biochem 2003;40:453-71.
10. Tripathi S, Ammini AC, Bhatia R, Gupta R, Berry M, Sarkar C, *et al*. Cushing's disease: Pituitary imaging. Australas Radiol 1994;38:183-6.
11. Sahdev A, Reznek RH, Evanson J, Grossman AB. Imaging in Cushing's syndrome. Arq Bras Endocrinol Metabol 2007;51:1319-28.
12. Shah NS, Goel AH, Nagpal RD, Menon PS. Cushing's disease: Management outcome in a tertiary care centre. J Assoc Physicians India 2006;54:919-22.
13. Chowdhury IN, Sinaii N, Oldfield EH, Patronas N, Neimann LK. A change in pituitary MRI protocol detects ACTH-secreting tumours in patients with previously negative results. Clin Endocrinol 2010;72:502-6.
14. Chambers EF, Turski PA, LaMasters D, Newton TH. Regions of low density in the contrast-enhanced pituitary gland: Normal and pathologic processes. Radiol 1982;144:109-13.
15. Scriba PC, Hacker R, Dieterle P, Kluge F, Hochheuser W, Schwarz K. ACTH-Bestimmungen in plasma aus dem Bulbus cranialis venae jugularis. Klin Wochenschr 1966;44:1393-98.
16. Corrigan DF, Schaaf M, Whaley RA, Czerwinski CL, Earll JM. Selective venous sampling to differentiate ectopic ACTH secretion from pituitary Cushing's syndrome. N Engl J Me 1977;296:861-62.
17. Brown RD, Van loon GR, Ortho DN, Liddle GW. Cushing's disease with periodic hormonogenesis: One explanation for paradoxical response to dexamethasone. J Clin Endocrinol Metab 1973;36:445-51.
18. Findling JW, Aron DC, Tyrrell JB, Shinsako JH, Fitzgerald PA, Norman D, *et al*. Selective venous sampling for ACTH in Cushing's syndrome. Ann Intern Med 1981;94:647-52.
19. Drury PL, Ratter S, Tomlin S, Williams J, Dacie JE, Rees LH, *et al*. Experience with selective venous sampling in diagnosis of ACTH-dependent Cushing's syndrome. Br Med J (Clin Res Ed) 1982;284:09-12.
20. Vonwerder K, Scriba PC. Jugular-vein sampling of ACTH. N Engl J Med 1977;297:730.
21. Lopez J, Barcelo B, Lucas T, Salame F, Alameda C, Boronat M, *et al*. Petrosal sinus sampling for diagnosis of Cushing's disease: Evidence of false negative results. Clin Endocrinol 1996;45:147-56.
22. Sweringen B, Katznelson L, Miller K, Grinspoon S, Waltman A, Dorer DJ *et al*. Diagnostic errors after inferior petrosal sampling. J Clin Endocrinol Metab 2004;89:3752-63.
23. Wiggam MI, Heaney AP, McIlarth EM, McCance DR, Sheridan B, Hadden DR, *et al*. Bilateral IPSS in the differential diagnosis of ACTH-dependent Cushing's syndrome: A comparison with other diagnostic tests. J Clin Endocrinol Metab 2000;85:1525-32.
24. Doppman JL, Chang R, Oldfield EH, Chrousos G, Stratakis CA, Nieman LK. The hypoplastic inferior petrosal sinus: A potential source of false negative results in petrosal sampling for Cushing's disease. J Clin Endocrinol Metab 1999;84:533-40.
25. Lefournier V, Martinie M, Vasdev A, Bessou P, Passagia JG, Labat-Moleur F, *et al*. Accuracy of bilateral inferior petrosal or cavernous sinuses sampling in predicting the lateralization of Cushing's disease pituitary microadenoma: Influence of catheter position and anatomy of venous drainage. J Clin Endocrinol Metab 2003;88:196-203.
26. Erickson D, Houston J, Young WF, Carpenter PC, Wermers RA, Bonelli FS, *et al*. Internal jugular vein sampling in ACTH-dependent cushing's syndrome: A comparison with inferior petrosal sinus sampling. Clinical Endocrinology 2004;60:413-19.
27. Ilias I, Chang R, Pacak K, Oldfield EH, Wesley R, Doppman J, *et al*. Jugular venous sampling: an alternative to petrosal sinus sampling for the diagnostic evaluation of ACTH-dependent Cushing's syndrome. J Clin Endocrinol Metab 2004;89:3795-800.
28. Obuobie K, Davies JS, Ogunko A, Scanlon MF. Venous thromboembolism following inferior petrosal sinus sampling in Cushing's disease. J Endocrinol Invest 2000;23:542-44.
29. Berkhout FTV, Croughs RJM, Kater L, Schuurman HJ, Gmelig Meyling FJ, Kooyman CD, *et al*. Familial Cushing's syndrome due to nodular adrenocortical dysplasia. A putative receptor-antibody disease? Clin Endocrinol 1986;24:299-310.
30. Wegienka LC, Wuepper KD, Komarmy LE, Forsham PH. Cushing's syndrome with adrenal medullary insufficiency and adrenal auto- antibodies. Lancet 1966;1:741-43.
31. Biswas K, Goyal R, Ammini AC, Karak AK, Sarkar C, Mishra NK, *et al*. Recurrent lymphocytic hypophysitis in a woman 27 years after subtotal adrenalectomy for hypercortisolism possibly of autoimmune origin. J Assoc Physicians India 2005;53:1066-69.
32. Kaltsas GA, Giannulis MG, Newell-Price JD, Dacie JE, Thakkar C, Afshar F, *et al*. A critical analysis of the value of simultaneous IPSS in Cushing's disease and the occult ectopic ACTH syndrome. J Clin Endocrinol Metab 1999;84:487-92.

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