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ACTH increment post total bilateral adrenalectomy for Cushing's disease: a consistent biosignature for predicting Nelson's syndrome

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Abstract

Purpose Nelson's syndrome (NS) is regarded as an aggressive complication of total bilateral adrenalectomy (TBA) for Cushing's disease (CD). This challenge may be addressed by using clinical criteria to guide frequency of neuroimaging to enable timely management of NS and also avoid unnecessary frequent imaging.

Methods All patients (n = 43) with CD subjected to TBA over 35 years at a tertiary care centre were included. NS was defined as a newly appearing or expanding (> 2 mm) pituitary adenoma with or without ACTH levels exceeding 500 pg/ml. Pre-and post-TBA parameters like clinical symptomatology, cortisol, ACTH and radiology were analysed for the prediction of NS.

Results NS developed in 39.5% (n = 17) patients with a median follow-up of 7 years. Half of them had new appearance, while rest had an expansion of pre-existing pituitary tumour. Majority (90%) had ACTH above 500 pg/ml. On Cox proportional hazards analysis, frequent discriminatory features of protein catabolism (≥ 4) (HR 1.15, CI 0.18, 7.06), proximal myopathy (HR 8.82, CI 1.12, 69.58) and annual ACTH increment of 113 pg/ml (HR 12.56, CI 1.88, 88.76) predicted NS. First post-operative year ACTH indices predicting NS included ACTH rise of 116 pg/ml and absolute ACTH of 142 pg/ml (sensitivity, specificity exceeding 90%). Annual ACTH increment exceeding 113 pg/ml, ≥ 4 discriminatory features and uncontrolled hypertension had the best overall prediction.

Conclusion Patients who developed NS had higher rebound rise of ACTH following TBA and a more severe disease phenotype at baseline. Consistent ACTH increment can be used as a marker for predicting the development of NS.

Keywords Nelson's syndrome · Cushing's disease · Discriminatory features · Proximal myopathy · ACTH

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Abbreviations

NS	Nelson's syndrome
CD	Cushing's disease
CS	Cushing's syndrome
TSS	Trans-sphenoidal surgery
TBA	Total bilateral adrenalectomy
ACTH	Adrenocorticotropic hormone
ONDST	Overnight dexamethasone suppression test
LDDST	Low dose dexamethasone suppression test
HDDST	High dose dexamethasone suppression test
IRMA	Immunoradiometric assay
ECLIA	Electrochemiluminescence assay
CV	Coefficient of variation
IQR	Interquartile range
SD	Standard deviation
UFC	Urinary free cortisol

Introduction

Cushing's disease (CD) is characterized by ACTH-dependent cortisol excess due to a corticotropinoma accounting for 70% of all endogenous Cushing's syndrome (CS) [1]. The treatment of choice for CD is trans-sphenoidal surgery (TSS). However, long-term outcomes following TSS shows disease persistence/recurrence in atleast one-third cases [2, 3]; the management of which involves repeat TSS, gamma knife radiosurgery or total bilateral adrenalectomy (TBA). Repeat TSS has lower chances of remission and higher rates of recurrence as well as peri-operative complications and hypopituitarism, as compared to primary TSS [4, 5]. Gamma knife or conventional radiotherapy leads to delayed resolution of hypercortisolism, mandates medical management in the interim period and has a definitive risk of hypopituitarism [6]. Medical management of hypercortisolism has variable efficacy, side effects and is theoretically, lifelong. TBA is therefore regarded as a third line therapy in these circumstances [4, 6–8]. Furthermore, there are instances of severe hypercortisolism ("catastrophic Cushing's") wherein an instantaneous control of the hypercortisolic state is desirable and in these patients, TBA is used as a first line therapy in a life-saving attempt [3, 4]. In both situations, TBA provides a rapid and unequivocal resolution of hypercortisolism with favourable long- term outcomes [8]. But, Nelson's syndrome (NS) is one of the important potential complications that requires dedicated long-term surveillance.

NS develops in response to loss of negative feedback following TBA in a patient with CD. Though originally reported by Nelson et al. [9], even after six decades, there are controversies in its definition, surveillance protocol and predictive factors which has led to a reported highly variable incidence varying from 0 to 47% [8]. Rising ACTH and Nelson's adenoma have been given equal weightage for the diagnosis of NS [10], but adenoma development may not always parallel increment in ACTH [11, 12]. The clinical significance of increased ACTH after adrenalectomy is resultant hyperpigmentation and rarely development of adrenal rest tumours while adenoma progression can be associated with visual deficits, cranial neuropathies, apoplexy, proliferation of ectopic adrenal rests [13–16] and rarely, malignant transformation [12]. Therefore, the goal of management in NS should be the early detection and management of the expanding corticotroph adenoma rather than rising ACTH, but this concept does not find a global consensus.

Available literature is inconsistent with respect to various predictive factors for the development of NS [10–13, 17, 18]. CD is characterised by preserved response to negative feedback by cortisol but at a higher set-point [19, 20]. Loss of this negative feedback following TBA leads to rise in ACTH and development of Nelson's adenoma. The magnitude of this change in feedback loop and consequent rise in ACTH may be a marker for predicting the development of NS.

The aim of the current study was to review the outcomes of TBA for CD with particular emphasis on NS. The study reports the incidence, presentation, management and predictors of NS.

Materials and methods

Study design

This patient cohort is from a single tertiary care centre in North India (Post Graduate Institute of Medical Education and Research, Chandigarh) over a period of 35 years from 1984 to 2019 with prospective follow-up from 2004 and retrospective data retrieval prior to that. The study was approved by the Institutional Ethics Committee (IEC/2017/85). Written informed consent for publication of their clinical details was obtained from the patients or relatives as applicable. Demographic, anthropometric, clinical, biochemical and imaging details were recorded prospectively.

Study participants

All patients with CD subjected to TBA were included. Indications for adrenalectomy included persistence of disease following TSS, presence of severe co-morbidities and non-localization of corticotropinoma. Baseline analysis was either noted prospectively as per standard protocol or from file records. Follow-up assessment included patient visits and file records.

Pre-TBA evaluation

Definitions and clinical parameters

Clinical features including discriminatory features of CS (due to protein catabolism) like proximal myopathy, striae, bruise, plethora and cuticular atrophy were recorded systematically. Proximal myopathy was defined as power less than 4/5 (MRC grade) and striae were considered when livid with maximum diameter exceeding 1 cm. Centripetal obesity was defined as a waist circumference exceeding 80 cm in females and 90 cm in males irrespective of BMI as per Asian criteria [21]. Generalized obesity was defined as BMI exceeding 25 kg/m^2 [22]. Diabetes and hypertension were documented by standard criteria [23, 24]. Prediabetes was defined on the basis of oral glucose tolerance test or any derangement in fasting (100–125 mg/dl), post prandial glucose value (140–199 mg/dl) and/or HbA1c (5.7–6.4%). Uncontrolled hypertension was defined as failure to lower blood pressure to 140/90 mmHg with optimal doses of 3 anti-hypertensives, including a diuretic (spironolactone). Uncontrolled diabetes was defined as non-attainment of HbA1c < 7% with multimodality management, including insulin. Dyslipidemia was defined as triglycerides exceeding 150 mg/dl, increased LDL and/or low HDL (< 40 for males, < 50 for females) [25]. Severe osteoporosis was defined as osteoporosis with any fragility fracture.

Investigation profile

Baseline cortisol and ACTH levels (08.00 h) were sampled after overnight fasting. 23.00 h plasma cortisol and ACTH were sampled in awake state after admission to the ward. Overnight dexamethasone suppression test (ONDST), low and high dose dexamethasone suppression tests (LDDST, HDDST) were performed as per the standard protocol [26]. Serum cortisol was assessed by ECLIA (electro-chemiluminescence-immuno-assay) from 2009 onwards and RIA prior to that. ACTH levels were measured by ECLIA (ELECSYS Roche Diagnostics, Germany) with intra and inter-assay CV of 1.4–2.8% and 2.3–6.4% respectively and RIA (Biosure technologies, Nivellis, Belgium) with intra-assay and inter-assay CV of 4.7 to 8% prior to that. The correlation co-efficient between both assays was 0.8 as has been noted in other studies comparing both assays [27, 28]. Urine free cortisol was not estimated till recently at our centre and hence its report was not available for all patients. Baseline biochemistry included plasma glucose, glucose tolerance test if not previously diagnosed with diabetes, fasting lipid profile, and electrolytes. DXA (Hologic) and/or quantitative CT (qCT) at baseline was performed to diagnose osteoporosis or osteopenia. Dorsal vertebral X rays were used to detect vertebral fractures. Contrast enhanced dynamic MRI sella (Siemens Magnetom) was done wherever possible. In subjects with a non-visualised pituitary tumour, BIPSS (Bilateral inferior petrosal sinus sampling) or CT thorax and abdomen/somatostatin receptor-based scintigraphy were performed to document the absence of an ectopic source of CS.

Post-TBA evaluation

All patients were given a bolus dose of 100 mg hydrocortisone followed by a 4 mg/hr infusion on the day of surgery. Doses were gradually tapered to oral hydrocortisone at 8–10 mg/m^2 or equivalent doses of prednisolone in cases where non-compliance due to multiple dosing or cost was expected. Fludrocortisone was supplemented at 50–100 µg/m^2. Intra- and post-operative complications, if any, were documented.

NS was defined as an expanding (2 mm increase from baseline) or newly appearing pituitary adenoma with or without an 08.00 h ACTH value exceeding 500 pg/ml, prior to steroid administration. Adrenal crisis was defined as hypocortisolism requiring admission, intravenous glucocorticoid administration or sudden death due to adrenal insufficiency as per verbal autopsy. The departmental protocol for follow-up of NS was as follows: measurement of 08.00 h ACTH prior to the morning dose of steroid, at 6 weeks, 3 months, 6 months and then annually. Dynamic MRI brain focusing on the pituitary-hypothalamic area was usually done at ACTH levels exceeding 500 pg/ml, in concordance with the international consensus [3]. Exceptions included patients with pre-op non-visualization of pituitary tumour in whom MRI was performed at an interval of 2–3 years following TBA based on clinical judgement, despite ACTH being lower than 500 pg/ml. The average annual rate of rise of ACTH was calculated for each patient. Values were extrapolated if any follow-up value was missing.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 22.0 software program (IBM Statistics 22.0). Data are presented as n (%), mean ± SD or median (quartile q25–q75), as appropriate. Qualitative variables were compared between the groups using Pearson $\chi 2$ test or Fisher's exact test. All quantitative parameters were checked for normality using the Kolmogorov–Smirnov test and classified as parametric and non-parametric. Student *t* test was used to compare the means of two groups for parametric data and Mann–Whitney *U* test for non-parametric data. Differences among groups were analysed using McNemar tests. In view of different times of development of NS for different patients, Cox proportional hazards model was used to compute the effects of covariates influencing the development of NS. Unadjusted and adjusted hazards ratio

(95% CI) was calculated for each covariate. A p-value < 0.05 was considered significant. Probability prediction was performed various parameters that emerged significant for development of NS. Derived receiver operating curves (ROC) were used to determine various ACTH indices at highest sensitivity and specificity.

Results

There were 186 patients of CD of which 45 were subjected to TBA. Evolution of NS was analyzed in 43 of them as the last two patients had a short follow-up (less than 6 months). The overall median duration of follow-up (n = 43) was 7 (1–12) years.

Table 1 Pre-TBA clinical characteristics of the cohort (n = 43)

Clinical parameter	Mean ± SD/median(IQR)
Mean age	32 ± 14 years
Median lag period before diagnosis	2 (1–4) years
% Females	67
Mean BMI	26.5 ± 4.6 kg/m^2
Centripetal obesity	81%
Menstrual irregularity	Oligomenorrhoea 60%, amenorrhoea 7%
Hirsutism	67%
Hyperpigmentation	61%
Discriminatory features of Cushing's syndrome	
Proximal myopathy	82%
Cuticular atrophy	73%
Striae	65%
Bruise	65%
Plethora	53%

Data are presented as mean ± SD, median (IQR) or n (%)

Pre-TBA evaluation of Cushing's disease

Baseline demographics and investigational parameters are summarized in Tables 1 and 2. Nearly 75% of the population was in the 2nd to 4th decades with a female preponderance (67%). Discriminatory features of CS owing to protein catabolism were prevalent in 50–80% patients. Proximal myopathy was a presenting manifestation in 40% of patients (Tables 1, 2). Broad, violaceous striae were noted in two-thirds of the cohort. Diabetes was present in nearly half and hypertension, osteoporosis as well as dyslipidemia in more than two-thirds of the cohort. Severe comorbidities included neuropsychiatric manifestations with suicidal ideation, intervertebral disc prolapse, fungal (aspergillus) as well as bacterial empyema and lumbosacral plexopathy/amyotrophy.

MRI of the pituitary-hypothalamic area was able to localise tumour in 78%, and three-fourths of these were microadenomas. Histopathological evidence of corticotropinoma was found in 88%. In the rest of the patients (n = 8), 4 developed NS on follow-up, thereby confirming that they had CD. The other 4 were also likely to have had CD in view of no clinical or imaging evidence of ectopic CS on follow-up and the fact that CD has a high pre-test probability in a given case of ACTH dependent CS.

Cortisol dynamics in response to dexamethasone suppression tests are summarized in Table 2. HDDST in our cohort showed non-suppressibility (to less than 50% of basal value) in 27% of the patients.

Pre-op medical management included ketoconazole in varying doses (400–1200 mg) for a variable duration (1 week-3 months) in nearly half of the patient population and etomidate in one patient (0.02 mg/kg/h). All patients were subjected to TBA and majority were subjected to a laparoscopic procedure. Primary TBA was attempted in 70% (severe comorbidities (n = 20), non-localisation of pituitary tumour (n = 8) or other causes like non-pneumatisation of sinuses (n = 2)). Secondary TBA following failed TSS was

Table 2 Pre-TBA biochemical and radiological characteristics of the cohort (n = 43)

Parameter	Mean ± SD/median (IQR)
08.00 h cortisol	34.5 ± 23.6 µg/dl
23.00 h cortisol	22.8 ± 5.6 µg/dl
Cortisol post overnight 1 mg dexamethasone suppression (ONDST)	29.3 ± 7.5 µg/dl
Cortisol post low dose (2 mg) dexamethasone suppression test (LDDST)	16.5 ± 8.4 µg/dl
Cortisol post high dose (8 mg) dexamethasone suppression test (HDDST)	8.3 ± 1.3 µg/dl
Non-suppressible cortisol on HDDST	27%
Pre-TBA 08.00 h ACTH	51 pg/ml (31–97)
Pre-TBA adenoma type	Microadenomas 61%, macroadenomas 30%, normal sella 9%

Data are presented as mean ± SD, median (IQR) or n (%)

ONDST overnight dexamethasone suppression test, *LDDST* low dose dexamethasone suppression test, *HDDST* high dose dexamethasone suppression test

done in 30% patients. Intra-operative period was uneventful except for hypertensive crisis in 1 patient. Postoperative complications included pneumonitis and acute respiratory distress syndrome (n = 3), pulmonary thromboembolism/deep vein thrombosis and paralytic ileus (n = 2 each) diabetic ketoacidosis, suture line sepsis and subpleural collection (n = 1 each).

Post-TBA evaluation of the development of Nelson's syndrome

NS developed in 17 out of 43 patients (39.5%) at a median duration of 7 (1–12) years of follow-up. 53% of these patients had expansion of pre-existing pituitary mass while 47% had new appearance of tumour. The median duration for the development of adenoma was 3 years. But, 18% patients developed NS at or after 10 years following adrenalectomy. In those patients that developed Nelson's, the pre-TBA distribution of adenoma showed 12% macroadenomas, 41% microadenomas and 47% normal sella. Median ACTH levels at time of diagnosis of NS were 1004 pg/ml (553–1652). There were 2 patients who had a Nelson's adenoma without ACTH exceeding 500 pg/ml. One was diagnosed with a Nelson's adenoma at an ACTH of 172 pg/ml, 6 years post-adrenalectomy. On further evaluation, he was found to have residual adrenal tissue. 43% of Nelson's adenomas were macroadenomas and 53% were microadenomas. The average rate of tumour growth was 2.3 mm/year and the median adenoma diameter was 8 (5.5–15) mm. Hyperpigmentation was present in all patients but symptomatic Nelson's adenoma was seen in only one patient who had an invasive macroadenoma causing multiple cranial neuropathies (3rd, 4th and 6th) due to cavernous sinus invasion. This index patient had a microcorticotropinoma at the age of 18 years, persistent disease following TSS for which she was subjected to TBA. She was lost to follow-up in the interim and presented with a compressive macroadenoma 6 years later, necessitating repeat TSS.

There was a weak correlation between rate of ACTH rise and Nelson's tumour volume ($r = 0.4$, $p = 0.28$) but none with tumour diameter or rate of tumour growth. There were 12 subjects in whom pituitary tumour was present at baseline (all except one had microadenoma), but they did not develop NS following TBA. Six of these did not display tumour progression, two patients received gamma knife prophylactically, and three (including the one with macroadenoma) died while one was lost to follow-up.

Definitive therapy was offered to all patients following a diagnosis of NS. Nine were subjected to TSS and seven were treated with gamma knife radiotherapy. One subject with NS was found to have fungal sinusitis, necessitating treatment with voriconazole post TSS. Another patient, who presented with secondary infertility, developed NS and is planned for gamma knife but is currently pregnant.

Prediction of Nelson's syndrome

The pre-operative parameters that could significantly predict the development of NS were the number of clinical discriminatory features of protein catabolism (≥ 4) in a given patient ($p = 0.02$), proximal myopathy as a presenting feature ($p = 0.03$) and hirsutism in females ($p = 0.03$) (Table 3). The presence of four or more discriminatory features yielded a positive predictive value of 53% and a negative predictive value of 71% for NS. Among those subjects who had three or less discriminatory features of protein catabolism, only 29% patients developed NS whereas 70% did not. Uncontrolled diabetes and hypertension were present more commonly in those who developed NS but this association only showed a trend towards significance ($p = 0.07$).

There was no difference between both groups with respect to age, gender, lag period, baseline BMD, hypokalemia, ketoconazole use or duration of follow-up. There was also no difference between both groups in terms of pre-op adenoma localisation, tumour dimensions, primary versus secondary adrenalectomy. Importantly, neither baseline cortisol levels nor those obtained following dexamethasone suppression tests (ONDST, LDDST) were significantly different in the patients that developed NS as compared to those that did not ($p > 0.05$) (Table 3). HDDST was non-suppressible in 27% of the cohort. One third of these had visualised adenomas (> 6 mm) and one third developed NS on follow-up. In the other one-third, CT thorax and abdomen did not reveal any ectopic source of CS nor did they develop features consistent with a primary ectopic source of ACTH during follow-up. However, there was an interesting association of NS with pre-operative ACTH levels. NS developed more in patients who had lower ACTH (median 51 pg/ml, 25–102) than in those who had higher levels at baseline (median 75 pg/ml, 31–101) ($p > 0.05$).

Post-operative factors that were significantly associated with NS included the first post-operative year absolute ACTH as well as ACTH increment ($p < 0.05$) (Table 4). Subsequently, the median annual increment of ACTH in any year was also higher in the subgroup who developed NS ($p = 0.01$). ROC analysis revealed a first year ACTH increment of 116 pg/ml and absolute ACTH of 142 pg/ml as predictive of NS with a sensitivity of 90% and specificity of 100% each. Similarly, a subsequent annual ACTH increment of 113 pg/ml/year and an absolute ACTH of 499 pg/ml had a sensitivity of 85% and specificity of 86% each ($p = 0.003$ for both) for the prediction of NS (Table 5).

Cox proportional hazards model showed the predictive ability of annual increment in ACTH exceeding 113 pg/ml (adjusted HR 12.56 CI 1.8, 88.7, $p = 0.01$), proximal muscle

Table 3 Pre-TBA predictors of Nelson's syndrome

Parameter	Nelson's (n = 17)	Non-Nelson's (n = 26)	p value
Age (years)	31.1 ± 10	30.4 ± 15	0.872
% Females	64	65	0.608
Lag period (years)	2.28 ± 1.96	2.68 ± 2.28	0.547
Striae	82%	17%	0.032*
Proximal myopathy as presenting feature	70%	30%	0.031*
Number (%) of patients having ≥ 4 discriminatory features of protein catabolism (n = 19)	10/17 (59%)	9/26 (35%)	0.025*
Hirsutism	75%	25%	0.031*
Uncontrolled diabetes (n = 15)	12 (80%)	3 (20%)	0.071
Uncontrolled hypertension (n = 20)	16 (80%)	4 (20%)	0.071
Mean 08.00 h cortisol (μg/dl)	33.8 ± 13.5	34.2 ± 13.2	0.921
Mean 08.00 h ACTH (pg/ml)	51 (25–102)	75 (31–101)	0.599
Mean cortisol post ONDST (μg/dl)	26.7 ± 15.8	27.2 ± 15.2	0.900
Mean cortisol post LDDST (μg/dl)	23.5 ± 16.1	23.8 ± 14.0	0.769
Median cortisol post HDDST (μg/dl)	8.5 (3.1–26.2)	7.9 (4.1–23.7)	0.858
Median adenoma size (mm)	5.5 (3–8)	8.5 (6–12)	0.475
Ketoconazole given	45%	55%	0.309

Data are presented as mean ± SD, median (IQR) or n (%)

*denotes significance of the given parameter ($p < 0.05$)

ONDST overnight dexamethasone suppression test, *LDDST* low dose dexamethasone suppression test, *HDDST* high dose dexamethasone suppression test

Table 4 Post-TBA predictors of Nelson's syndrome

Parameter	Nelson's (n = 17)	non-Nelson's (n = 26)	p value
Mean paired adrenal weight (g)	21 ± 15	28 ± 17	0.286
First year increment in ACTH (pg/ml)	443 (302–823)	46 (23–96)	0.005*
ACTH at 1 year post adrenalectomy (pg/ml)	501	97	0.002*
Median annual rate of rise of ACTH (pg/ml/year)	420	51	0.002*
Prior TSS	40%	60%	0.400
Post op diabetes mellitus	16%	17%	0.990
Post op hypertension	17%	20%	0.563

Data are presented as mean ± SD, median (IQR) or n (%)

*denotes significance of the given parameter ($p < 0.05$)

TSS Trans-sphenoidal surgery

Table 5 Derived ROC values for various ACTH indices

Parameter	Value at maximum sensitivity	Value at maximum specificity	Value at best possible sensitivity and specificity	Sensitivity (%)	Specificity (%)	AUC	p value
First year absolute ACTH	121 pg/ml	288 pg/ml	142 pg/ml	90	100	1.000	0.003*
First year increment in ACTH	126 pg/ml	169 pg/ml	116 pg/ml	90	100	0.960	0.00*
Absolute ACTH at time of occurrence of NS	47 pg/ml	572 pg/ml	499 pg/ml	85	86	0.912	0.004*
Average annual increment in ACTH	54 pg/ml/year	175 pg/ml/year	113 pg/ml/year	85	86	0.905	0.015*

ROC receiver operating curves, *AUC* area under curve, *NS* Nelson's syndrome

*denotes significance of the given parameter ($p < 0.05$)

Values expressed as ACTH indices at best possible sensitivity, specificity and both sensitivity and specificity

weakness as a presenting manifestation (adjusted HR 8.82 CI 1.1, 69.5, p = 0.03), uncontrolled hypertension (adjusted HR 1.14 CI 0.2, 5.3, p = 0.85) and the presence of 4 or more discriminatory features of protein catabolism (adjusted HR 1.15 CI 0.1,7.0, p = 0.88) for NS (Table 6). Due to expected multicollinearity between various ACTH indices, only annual ACTH increment was chosen for further analysis.

Probability analysis revealed a combination of ≥ 4 discriminatory features, uncontrolled hypertension and annual increment in ACTH (90%) (Table 7) as having highest predictive ability for NS.

Discussion

Nelson's syndrome is an outcome of loss of negative feedback due to withdrawal of hypercortisolemia following TBA. Our study demonstrates that patients who developed NS showed a severe disease phenotype with frequent presence of features of protein catabolism and uncontrolled metabolic co-morbidities pre-TBA, as well as greater rebound rise in ACTH post-TBA, despite having comparable circulating cortisol levels to those who did not develop NS. The ACTH increment was consistent, suggesting its utility in predicting NS, especially in those patients who have a severe disease phenotype pre-TBA. The most frequent indications for TBA in patients with CD include persistent disease following TSS, presence of severe comorbidities and adenoma non-localisation [6–8, 29, 30]. 24% (n = 45) of the patients with CD underwent TBA as a secondary or primary procedure at our centre over the period of 35 years and 43 of them were analysed for the NS.

HDDST in our patients was non-suppressible in approximately one-fourth of the patients. However, given the pre-test probability of CD in patients with ACTH dependent CS being 80–90%, the utility of HDDST is probably questionable in reliably diagnosing CD. The efficacy and effectiveness of HDDST in diagnosing CD remains low (specificity 66% and similar range of ACTH suppression in both CD and ectopic causes) and it adds little to other clinical and biochemical parameters in the differential diagnosis of ACTH dependent CS [31]. Moreover, two-thirds of these had definite CD either due to presence of adenomas in them or the development of NS. Only 4 continued to have occult source but even after a mean follow-up of 13.5 years in these patients, the ectopic source was not seen, further strengthening the diagnosis of CD.

The criteria to diagnose NS used in the literature include either 08.00 h ACTH levels ≥500 pg/ml and/or presence of pituitary adenoma [10]. However, the more significant target of therapy in NS should be the tumour as Nelson's adenoma is much more of a threat due to compressive features, proliferation of adrenal rests and rarely malignant transformation [12–16]. Hence in the present study, a clinically relevant definition of NS mandating the presence of Nelson's adenoma with or without ACTH levels exceeding 500 pg/ml was used uniformly. NS developed in nearly 40% of the cohort at a median follow-up for 7 years. Previously large cohorts have shown variable incidence of NS (0–50%, median 21%) attributable to the lack of a uniform definition, alteration in natural history of the disease due to prior

Table 6 Association of clinical and biochemical parameters with the development of Nelson's syndrome on follow-up using Cox proportional hazards regression analysis

Parameter	Unadjusted HR	95% CI	p value	Adjusted HR
≥ 4 discriminatory features of Cushing's syndrome	1.15	0.18–7.06	0.88	1.15
Uncontrolled hypertension	1.12	0.24–5.30	0.85	1.14
Proximal myopathy	9.96	1.12–69.58	0.03*	8.82
Average annual increment in ACTH ≥ 113 pg/ml/year	12.26	1.81–88.76	0.01*	12.56

*denotes significance of the given parameter ($p < 0.05$)

Table 7 Multivariate probability analysis for prediction of Nelson's syndrome

Combination of various parameters significant for prediction of NS	Probability of Nelson's (%)	p value
≥ 4 discriminatory features of Cushing's syndrome + proximal myopathy	74	0.007*
≥ 4 discriminatory features of Cushing's syndrome + proximal myopathy + uncontrolled hypertension	75	0.012*
≥ 4 discriminatory features of Cushing's syndrome + proximal myopathy + average annual increment in ACTH	82	0.010*
≥ 4 discriminatory features of Cushing's syndrome + uncontrolled hypertension + average annual increment in ACTH	90	0.015*

*denotes significance of the given parameter ($p < 0.05$)

TSS/radiotherapy and improvement in imaging modalities with time in different studies [10–13]. The incidence in this study was relatively higher than globally reported, possibly because of long duration of follow-up. However, our figures are comparable to large single or multicenter studies, especially when corticotroph tumour progression (regarded as the conceptual revision of the arbitrary definition of NS) was taken into account [3, 11, 12, 31, 32].

The time-interval for neuroimaging to detect Nelson's adenoma is not well-defined. Most of the patients in our cohort underwent neuroimaging once ACTH peaked more than 500 pg/ml in accordance with literature [11]. Hyperpigmentation was common due to high ACTH levels but compressive features were infrequent, probably due to active surveillance. Our median ACTH at time of diagnosis of NS was 1004 pg/ml which is comparable to other series with average values between 450 to 1100 pg/ml. However, one patient had ACTH of 172 pg/ml at the time of diagnosis of NS, which was due to an adrenal remnant. This happened possibly because following TBA, a total loss of negative feedback resulted in the growth of Nelson's adenoma but its growth was restricted due to the residual adrenal leading to lower ACTH rise as compared to other patients, depicting the dynamic interaction between cortisol and ACTH. There was no correlation between ACTH increment and rate of adenoma growth or tumour volume, in concordance with previous reports which show a poor correlation between tumour dimension and ACTH [11]. Moreover, most of these tumours were microadenomas and all the three dimensions were frequently not measurable.

The predictors of NS have been inconsistent and controversial. Pre-operative ACTH levels have been shown by some to predict NS but the most validated parameter is post-operative ACTH [10, 11, 33]. Age, lag period, pre-op adenoma visualization, histopathology showing adenoma, baseline serum or urinary free cortisol, non-suppressible HDDST, long duration of follow-up, subnormal glucocorticoid replacement and external beam or gamma knife radiotherapy have been found to be significant predictors in some studies while in others they have not [10, 11, 13, 17, 18, 33–35]. A recent multicentric study identified the usage of multimodality therapy as the lone predictive factor in the development of NS, implying that severe the disease, the more likely it is to develop NS [12]. Our study is in accordance with these findings as those who had a severe disease phenotype were more likely to develop NS.

In our study, the pre-TBA parameters that predicted NS were severe disease phenotype substantiated by more frequent presence of discriminatory features of protein catabolism (≥ 4), striae and proximal myopathy as the presenting symptom, indicating more cortisol sensitivity in these patients as proximal myopathy not merely as a sign but also as a presenting symptom indicates more severe disease. However, serum cortisol levels were not different from those who did not develop NS.

Moreover, patients who developed NS had a lower pre-operative ACTH (albeit not significant). Post-TBA, they had a significantly higher first year as well as subsequent annual rise in ACTH following adrenalectomy. Although RIA was used for ACTH estimation prior to ECLIA, a good correlation exists between both and the RIA is useful at higher ACTH concentrations as reported earlier [27, 28]. A first-year increment in ACTH from baseline (116 pg/ml), the first year absolute ACTH (142 pg/ml) and the subsequent average annual increment in ACTH (113 pg/ml) significantly predicted NS. These parameters are hitherto undescribed except the first-year rise in ACTH. Lower baseline ACTH and a higher rebound rise in ACTH following loss of negative feedback due to adrenalectomy was a consistent feature in those who developed NS. We thereby propose monitoring of ACTH and using cutoffs as listed to predict timely management of NS, especially in those with severe disease phenotype. Nelson's and Cushing's adenoma are genetically same, yet the former is perceived as being more aggressive, probably due to complete loss of negative feedback following TBA [36, 37]. In our study, 43% of patients had macroadenomas as neuroimaging was performed only when ACTH levels crossed 500 pg/ml, in accordance with existing literature [10], with one patient even having a compressive macroadenoma. On the other hand, neuroimaging guided by the severity of symptomatology and annual increment of ACTH could have aided earlier diagnosis of the evolving tumour while still in the micro-corticotropinoma stage so as to minimise perioperative morbidities and the risk of hypopituitarism. This points towards the necessity of earlier and incremental ACTH cutoffs not only in the first post-operative year but also subsequently, which can guide the frequency of neuroimaging.

The current study addresses this precise need by providing ACTH increment cutoffs which can enable early detection and treatment and prevent complications like visual defects, apoplexy or rarely, pituitary carcinoma [12, 16]. We also postulate a probable role of innate cortisol sensitivity in an individual, manifested by more severe disease phenotype (peripheral sensitivity) and lower pre-operative ACTH with greater rebound rise in ACTH following TBA (central sensitivity), in predicting NS. Circadian (by CRH) and/or ultradian (possibly by glucocorticoid feedback) rhythm disturbances may also have a role in the genesis of NS. However, these are only clinical clues and robust molecular-level evidence is lacking at present. Well-designed studies at the molecular/tissue level in larger patient cohorts can certainly shed more light on this relatively novel concept.

The strengths of this study include patient number, long duration of follow-up, homogenously defining NS with presence of an adenoma and derivation of various ACTH cut-off

which could be used as markers for the early detection of NS. Limitations include lack of urinary free cortisol in all patients and lack of an in-vitro assessment of tissue responsiveness to cortisol in these patients.

Conclusion

Increasing ACTH levels following TBA predict the development of Nelson's adenoma which should be regarded as the defining characteristic of NS. Rate of rise of ACTH following TBA should be employed in guiding the frequency of neuroimaging for early diagnosis and management of NS especially in those with a more severe disease phenotype.

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Author contributions LD did data compilation and analysis, drafted the manuscript, edited it and reviewed the literature. AB edited the manuscript and supervised patient management. RP was involved in interpretation of data and editing the manuscript. PD edited the manuscript and supervised patient management. SKB supervised patient management. CA provided neuro-radiological expertise in terms of imaging interpretation and/or inferior petrosal sinus sampling. SK, RM and ARB did adrenal surgery. UNS performed histopathological analysis. SSD did pituitary surgery. RW conceived the study, managed the patients, collected the data, performed interpretation of data and statistical analysis and edited the manuscript.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Consent to participate Written informed consent for enrollment was obtained from participants or their relatives as applicable.

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