Original Article

A Cross-Sectional Study on Comparison of Serum Cortisol Concentration Measured by Chemiluminescent Immunoassay in Four Different Automated Analyzers in a Variety of Adrenal Disorders

Neeti Agrawal, Anirban Sinha, Partha P. Chakraborty, Rana Bhattacharjee, Avivar Awasthi[1], Animesh Maiti

Department of Endocrinology and Metabolism, Medical College, Kolkata, West Bengal, [1]Department of Endocrinology, Kasturba Medical College, Manipal, Karnataka, India

Abstract

Introduction: Cortisol concentration is often estimated by competitive chemiluminescent immunoassays, which are prone to interference due to cross-reactivity. The extent of interference is inconsistent across different adrenal disorders and among different analyzers. Although liquid chromatography-mass spectrometry (LC-MS/MS) possesses better analytical specificity, it is not widely available. We aimed to compare cortisol values measured across four different analyzers across different adrenal disorders. **Methods:** Cortisol concentrations were measured in the serum of patients with congenital adrenal hyperplasia (CAH) (n = 12), primary adrenal insufficiency (PAI) (n = 11), endogenous Cushing's syndrome (CS) (n = 6), adrenal incidentaloma (AI) (n = 8), and healthy subjects (HS) (n = 10) in the following analyzers: Advia Centaur XP (Siemens) (SACXP), Immulite1000 (Siemens) (SI1000), Cobas e411 (Roche) (RCe411), and Architect (Abbott)(AA). **Results:** In CAH patients, a poor agreement was observed between SAC XP [median (IQR) 14.6 (4.7) µg/dL] and RC e411 [median (IQR) 4.6 (3.9) µg/dL] [ICC: −0.016, (−0.55, 0.55)]. The correlation was also poor between SAC XP and SI 1000 [ICC: 0.00, (0.558, 0.551)] as well as between SAC XP and AA [ICC: 0.089, (−0.488, 0.612)]. The agreement was good between RCe411, SI 1000 [median (IQR) 9.6 (3) µg/dL], and AA [median (IQR) 5.1 (3.4) µg/dL] platforms in the same group. Measured cortisol values correlated well across all analyzers in PAI, CS, AI, and HS. **Conclusions:** Cortisol concentration demonstrated variable agreement among different analyzers in different adrenal disorders. In CAH, cortisol values measured by SAC XP poorly correlate with RC e411, SI 1000, and AA platforms. Reassessment in another analyzer therefore would be prudent to avoid devastating consequences of unrecognized hypocortisolism in this subset of patients. A good concordance was observed among platforms in other conditions.

Keywords: Analyzer, chemiluminescent immunoassay, congenital adrenal hyperplasia, cortisol, liquid chromatography-mass spectrometry

Introduction

Serum and/or urine cortisol measurement is recommended during the evaluation of patients with suspected Cushing's syndrome (CS), adrenal insufficiency, and those with adrenal incidentaloma (s) (AI).[1-3] Accurate estimation of serum cortisol concentration is important for decision-making in all these conditions. Liquid chromatography-mass spectrometry (LC-MS/MS) is considered the gold standard for steroid measurement, including cortisol. However, it is labour-intensive, requires expertise, is expensive, and not widely available. A vast majority of the laboratories, therefore, measure cortisol by chemiluminescent immunoassay (CLIA).

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Address for correspondence: Dr. Rana Bhattacharjee, Department of Endocrinology and Metabolism, Medical College, Kolkata, West Bengal, Super Specialty Block (SSB), 4th Floor 88 College Street, Kolkata – 700 073, West Bengal, India.
E-mail: dr.r.bhatta@gmail.com

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Such immunoassays (IAs), performed on an automated platform, are convenient, rapid, relatively inexpensive, and require a smaller sample volume.[4] However, as IAs, including CLIA, are not structure-based, they are prone to exhibit positive interference due to cross-reactivity with other structurally similar steroids.[5] This happens due to the non-specific nature of the anti-cortisol antibodies used in such assays. As a result, estimated cortisol in CLIA may be falsely elevated, leading to diagnostic and therapeutic misadventures. The cross-reactivity of cortisol with other compounds is directly related to the extent of structural similarity of that molecule with cortisol and its circulatory concentration.[6] The interfering compounds can either be endogenous intermediates of steroid biosynthesis like cortisone, 11deoxy-cortisol (11DOC), 21deoxy-cortisol (21DOC), 17hydroxy-progesterone (17OHP), corticosterone, 11deoxy-corticosterone (11DOCS), or exogenous steroids. Under normal physiologic conditions, endogenous steroids circulate in much smaller concentrations compared to cortisol. However, their concentrations in serum may increase manifold in certain adrenal disorders such as CAH, resulting in an overestimation of serum cortisol, when measured by CLIA. Furthermore, the sources of anti-cortisol antibodies differ in different platforms. The estimated cortisol value, therefore, depends on the nature of the underlying disorder and the type of analyzer used.

Though such interference seems more relevant in CAH, a false elevation of serum cortisol concentration might alter clinical decision-making in other conditions as well. For instance, chronic adrenocorticotropic hormone (ACTH) excess in ACTH-dependent CS can increase the concentration of 11DOCS in circulation. Precursors of cortisol synthetic pathways like 17OHP may accumulate in the serum of patients with adrenocortical carcinoma (ACC). This probably occurs due to inefficient steroidogenesis following progressive dedifferentiation of these tumours.[7]

The present study was, therefore, conducted to compare serum cortisol concentrations measured by IAs in four different analyzers in a variety of adrenal disorders. In addition, healthy participants were also included to determine whether cortisol measurements by IAs vary among different analyzers in non-pathological states.

Materials and Methods

This was a single center, hospital-based, cross-sectional study conducted between February 2020 to March 2023.

Study cohort

Consecutive patients with suspected adrenal disorders were subjected to a detailed history, thorough physical examination, and appropriate baseline investigations. Diagnosis of CAH was considered based on clinical evidence of mucocutaneous hyperpigmentation, hyperandrogenism/genital ambiguity in 46, XX or gonadotropin-independent precocious puberty (GIPP) in 46, XY individuals along with biochemical parameters such as low morning serum cortisol (<5 μg/dL), elevated plasma ACTH concentration above two times the upper limit of reference range, high or suppressed plasma renin activity (PRA) [in 21-hydroxylase deficiency (21-OHD) or 11-beta-hydroxylase deficiency (11β–OHD), respectively], elevated 17 OHP (in 21-OHD), and increased androgens. Patients with morning serum cortisol levels between 5–18 μg/dL and plasma ACTH <300 pg/mL underwent a cosyntropin stimulation test (CST), while those with cortisol concentration between 5–18 μg/dL and plasma ACTH greater than 300 pg/mL did not. Similarly, in the primary adrenal insufficiency (PAI) group, a low morning serum cortisol (<5 μg/dL) along with elevated plasma adrenocorticotropic hormone (ACTH) concentration above two times the upper limit of the reference range established the diagnosis. Individuals with cortisol levels between 5–18 μg/dL underwent CST, depending upon the plasma ACTH concentration. Serum cortisol level of < 18 μg/dL at 60 minutes of CST suggested adrenal insufficiency. Patients with discriminating features of CS initially underwent morning serum cortisol assessment to exclude exogenous CS. A morning cortisol value of < 5 μg/dL suggested exogenous CS. Endogenous hypercortisolism was then confirmed by following tests: elevated "sleeping" midnight serum cortisol (>1.8 μg/dL) and a morning serum cortisol value of greater than 1.8 μg/dL, post 1 mg overnight dexamethasone administration, and those patients were included in CS group. Patients in whom adrenal masses were discovered incidentally on imaging, which was performed as a part of the evaluation of non-adrenal pathology, comprised the AI group. An overnight dexamethasone suppression test (ONDST) was performed to determine the autonomous cortisol secretion from AI. ONDST-cortisol level of more than 1.8 μg/dL indicated autonomous cortisol secretion from the mass. Patients with a history of exogenous glucocorticoid administration in any form within the last six months were excluded. In addition, patients with chronic kidney disease (CKD) and conditions prone to alter cortisol binding globulin (CBG) levels like pregnancy, chronic liver disease, and oral contraceptive medication use were excluded. Due to potential interference with biotin in IA, patients on multivitamins were also excluded. Study subjects were divided into the following five groups: (I) CAH (n = 12), (II) PAI other than CAH (n = 11), (III) endogenous CS (n = 6), (IV) AI (n = 8), and (V) healthy subjects (HS) (n = 10). All patients in the first four groups were treatment naive. Healthy participants who were not on any medication, known to alter cortisol metabolism or affect total cortisol measurement, were recruited as control (group V).

Sample collection

Blood samples were collected in plain tubes (without any anticoagulant) in the morning (between 8:00 and 9:00 hours) by venipuncture after an overnight fast. Samples were allowed to stand for 20–30 minutes, followed by centrifugation at 3000 rpm for 10 minutes. The supernatant fluid was then analyzed for serum cortisol levels in groups I, II, and V. Similarly, cortisol concentrations were estimated in groups III (endogenous CS) and IV (AI) but following ONDST. For ONDST, 1 mg of dexamethasone was administered under supervision in an oral form between 23:00 and 00:00 hours.

Cortisol was measured in the blood sample obtained on the following morning between8:00 and 9:00 hours. For each group, aliquots from same cortisol samples were measured in different analyzers. For ACTH measurement, a blood sample was drawn in a prechilled syringe and EDTA-containing vial. The sample was transported over an ice pack to the laboratory, cold centrifuged, and immediately analyzed.

Assay platforms

The serum cortisol was measured simultaneously in each sample by solid-phase competitive binding IA in four automated analyzers: Advia Centaur XP (Siemens Healthcare Diagnostics, Erlangen, Germany) (SAC XP), Elecsys cortisol II (Cobas e411, Roche Diagnostics GmbH, Mannheim, Germany)(RCe411), Immulite 1000 (Siemens Healthcare Diagnostics) (SI 1000), and Architect i1000 SR (Abbott, USA) (AA). Among them, SAC XP, SI 1000, and AA analyzers utilized CLIA, while the RCe411 analyzer used electrochemiluminescent IA (ECLIA). The intra-assay coefficient of variation (CV) for SAC XP, SI 1000, AA, and RC e411 analyzers was 2.89–3.09%, 5.8–7.8%, 2.4–5.5%, and 1.9–2.6%, while inter-assay CV was 3.07–3.83%, 6.3–8.4%, 3.2–6.2% and 3.2–3.8%, respectively. Plasma ACTH was measured by ECLIA in Roche Cobas e601 fully automated analyzer which has intra-assay CV of 3.5–5.4% and inter-assay CV of 4.2–5.1%. Serum 17OHP was measured at 8:00 hours, using either enzyme-linked immunosorbent assay (ELISA) or CLIA.

Statistical methods

Due to a dearth of studies investigating cortisol assay interference across various adrenal disorders, the sample size was not pre-determined, and a convenient sampling approach was adopted. The reliability of measured cortisol values among the four analyzers was assessed using the intraclass correlation coefficient (ICC), specifically the two-way mixed effects model (ICC 3,1). The type of coefficient employed was the single measure, and absolute agreement was defined as the criterion. Additionally, Bland–Altman plots were utilized to elucidate the magnitude of agreement between specific pairs of analyzers. For statistical analyses, JASP (Version 0.17.2.1), a statistical software developed by the University of Amsterdam, was used.

Ethical aspects

The study was approved by the institutional ethical committee (letter no. MC/KOL/IEC/NON-SPON/600/01/2020 dated 25/01/2020), Medical College, Kolkata. Written informed consent (and assent where applicable) was obtained from all participants. The procedures in the study followed the guidelines laid down in Declaration of Helsinki 1964.

Results

Baseline characters of the groups are summarized in Table 1.

Table 1: Demographic parameters of study participants in each category

	CAH	PAI	CS	AI	Control
Number of participants	12	11	6	8	10
Age [Median (IQR)]	4 (15.9)	25 (49.5)	31.5 (23.5)	54 (8.5)	28 (23)
Gender (M/F)	5/7 a	7/4	2/4	1/7	6/4

aGender in the CAH group refers to the gender of rearing

Group I (CAH) (n = 12)

Four (33.3%) patients presented with the typical salt-wasting crisis with failure to thrive, dehydration, hyponatraemia, and hyperkalaemia, suggesting classic salt-wasting CAH due to 21 hydroxylase deficiency (21-OHD). Five (41.7%) patients were diagnosed with the simple virilizing form of 21-OHD, and all of them had subclinical aldosterone deficiency as evidenced by elevated PRA. Non-classic CAH was diagnosed in one patient who presented with primary amenorrhea and had clinical and biochemical features of hyperandrogenism along with raised 17OHP concentration. The remaining two had 11β–OHD.

Six (50%) patients had morning serum cortisol concentration of less than 5 µg/dL when measured with RC e411. However, all of them exhibited significantly higher levels when the same samples were measured with SAC XP, and none of the values were below 5 µg/dL. In intraclass correlation (ICC) analysis, the agreement between SAC XP [median (IQR) 14.6 (4.7) µg/dL] and RC e411 [median (IQR) 4.6 (3.9) µg/dL] was found to be poor for morning cortisol [ICC (3,1) = −0.016, 95% CI = −0.55, 0.55]. Similarly, the correlation was also poor between SAC XP and SI 1000 [median (IQR) 9.6 (3) µg/dL] [ICC (3,1) =0.00, 95% CI = 0.558, 0.551] as well as between SAC XP and AA [median (IQR) 5.1 (3.4) µg/dL] [ICC (3,1) =0.089, 95% CI = −0.488, 0.612]. While, good agreement was observed among RC e411, SI 1000, and AA analyzers. A low ICC indicates low agreement between SAC XP and RCe411, SI 1000, and AA; however, it could have also occurred due to a small sample size.

Group II (PAI other than CAH) (n = 11)

In this group, diverse etiologies of PAI were encountered. Three (27.2%) patients belonged to the paediatric age group with the genetically confirmed diagnosis of familial glucocorticoid deficiency (FGD) due to nicotinamide nucleotide transhydrogenase (NNT) mutation, Allgrove syndrome, and X-linked adrenoleukodystrophy. Among the adult population, the most common cause of PAI was disseminated histoplasmosis or tuberculosis. The most common presenting complaint was recently developed mucocutaneous hyperpigmentation which was observed in nine (81.8%) patients. Biochemically, 10 out of 11 individuals had basal serum cortisol levels less than 18 µg/dL with plasma ACTH concentrations more than 300 pg/mL. As the ACTH level of more than 300pg/mL represents the maximum stimulus for cortisol secretion, performing a CST had no additional benefit in that setting.[1] One patient having serum cortisol less than 18 µg/dL and an ACTH level of 150 pg/mL failed to show cortisol value ≥ 18 µg/dL, post CST in all platforms. The cortisol values were concordant when measured by RC e411 [median (IQR) 3.4 (5.8) µg/dL]

and SAC XP [median (IQR) 3.6 (1.6) µg/dL]. The agreement among all four platforms was excellent [ICC (3,1) =0.954, 95% CI = 0.895, 0.985].

Group III (Endogenous CS) (n = 6)

One (16.7%) patient had ACC and another had ectopic ACTH-secreting CS due to bronchogenic carcinoma. One patient had primary pigmented nodular adrenal disease (PPNAD) as a cause of CS. The remaining three (50%) patients had a corticotrophin secreting pituitary adenoma. None of the patients was on metyrapone therapy. The correlation among all four assays was excellent for ONDST-cortisol {SAC XP [median (IQR) 28.1 (35.6) µg/dL], RCe411 [median (IQR) 26.4 (24.1) µg/dL], SI 1000 [median (IQR) 30 (24.4) µg/dL], AA [median (IQR) 26.3 (19.1) µg/dL]}[ICC (3,1) =0.896, 95% CI = 0.738, 0.97] in all of them.

Group IV (AI) (n = 8)

Among patients with AI, one individual had an ONDST-cortisol level of more than 5 µg/dL while the other two patients had ONDST-cortisol levels between 1.8–5 µg/dL as measured by all four platforms. Since those patients did not exhibit any clinical evidence of cortisol excess, they can be categorized as mild autonomous cortisol secretion (MACS)-2 and MACS-1, respectively.[8] For the remaining five patients, ONDST-cortisol levels were consistently below 1.8 µg/dL, and these results were in agreement across all platforms {SAC XPRCe411 [median (IQR) 1.5 (1.7) µg/dL], RCe411 [median (IQR) 1.6 (2) µg/dL], SI 1000 [median (IQR) 2 (2.2) µg/dL], AA[median (IQR) 1.5 (1.8) µg/dL]}.The correlation among these four assays was excellent [ICC (3,1) =0.966, 95% CI = 0.899, 911].

Group V (healthy control) (n = 10)

The agreement among all four platforms was good for basal cortisol in controls {SAC XP [median (IQR) 15 (3.1) µg/dL], RCe411 [median (IQR) 13.9 (2.3) µg/dL], SI 1000 [median (IQR) 13.9 (3.7) µg/dL], AA [median (IQR) 11.8 (2.5) µg/dL]} (ICC (3,1) =0.727, 95% CI = 0.483, 0.903).

Serum cortisol values in different groups are summarized in Table 2. Bland–Altman plots are provided in the Supplementary Section.

Discussion

The most common cause of CAH is a defect in the CYP21A2 followed by CYP11B1 gene.[9] The clinical presentation of classic 11β–OHD usually differs from 21OHD with respect to hypertension with/without hypokalaemia.[10] Low serum cortisol is the biochemical hallmark of CAH, irrespective of aetiology.

When serum cortisol values were compared between analyzers, we found that values measured by SAC XP had poor agreement with the other three platforms with the highest cortisol values obtained from the SAC XP analyzer. An earlier study conducted in metyrapone-treated patients, found that among different IAs, SAC XP and first-generation Roche E170 were particularly prone to overestimate serum cortisol concentration.[11] This was attributed to the differences in the extent of cross-reactivity between these analyzers. Depending upon the aetiology of CAH, whether 21OHD or 11β–OHD, one or more of the precursor hormones (17OHP, 21DOC, 11DOC, 11DOCS) circulate in very high concentration.[12] All these compounds cross-react with anti-cortisol antibody resulting in elevated measured concentration of cortisol. Compounds with strong cross-reactivity (more than 5%) had two-dimensional similarities of ≥ 0.867 to cortisol in the Roche Elecsys Cortisol assay.[6] Consequently, clinically significant cross-reactivities due to elevated concentrations of 21DOC and 11DOC occur in 21OHD and 11β–OHD, respectively. Besides structural similarity and concentration of the cross-reacting compounds, cross-reactivity also depends on the specificity of the anti-cortisol antibody used in a particular assay. A previous study, which assessed serum cortisol with two different radioimmunoassay kits in children with CAH due to 21OHD and adults on metyrapone therapy, noticed significant differences in the cortisol values measured by two kits. In infants with or without CAH, 24.6% had low cortisol concentrations with kit A versus 41.5% with kit B ($P < 0.05$, 17% discrepancies).[13] Furthermore, 11DOC concentrations had a significant impact on cortisol values measured by kit A. The source and nature of anti-cortisol antibodies in different analyzers vary. Hence, the degree of cross-reactivity also varies from analyzer to analyzer even for the same concentration of a particular compound in the sample. The user manuals of these analyzers provide data on the percentage of cross-reactivity for various compounds. Cross-reactivity for 17OHP is 1.4%, 0.08%, and 0.6% in SAC XP, RCe411, and AA platforms, respectively, when added in a concentration of 1000 µg/dL.[14-16] On the contrary, no cross-reactivity has been demonstrated in the SI 1000 platform even at a concentration of 400 µg/dL.[17] Among different endogenous compounds, maximum cross-reactivity is observed for 11DOC; 18.3% in SAC XP followed by 4.9% and 1.9% in RCe411 and AA, respectively. This is important in patients with 11β–OHD. In our CAH cohort (group I), two (16.7%) patients, who had hypertension at the initial visit, were reared as males and presented with GIPP and mucocutaneous hyperpigmentation. Serum cortisol concentrations were 30.6 µg/dL and 14.66 µg/dL in SAC XP while 3.04 µg/dL and 1.9 µg/dL when measured in RCe411 in those two boys, respectively. Primary glucocorticoid resistance (PGR) mimics 11β–OHD in many aspects except serum cortisol, which is high in the former and low in the latter condition. Thus, patients with 11β–OHD might be misdiagnosed as PGR, if serum cortisol is measured in the SAC XP platform.

Cross-reactivity for 21DOC is 10.3% and 2.4% in SAC XP and RCe411, respectively. This is particularly relevant in patients with suspected 21OHD. Interestingly, significant cross-reactivity of 17OHP and 11DOC with cortisol has not been detected in the SI 1000 analyzer. In the CAH cohort (group I), SAC XP yielded significantly higher baseline cortisol values compared to other platforms, and none of the values were less than 5 µg/

Table 2: Serum cortisol concentrations across four platforms and five groups

SAC XP	RCe411	SI 1000	AA
8–9 am serum cortisol values (mcg/dL) in group 1 (CAH)			
26.22	0.78	1.14	0.8
20.84	1.64	ND	ND
24.26	3.5	ND	ND
30.6	3.04	ND	7.5
9.2	6.27	8.76	5.8
14.66	1.9	6.69	5
25.7	5.74	10	ND
8.74	6.18	ND	5.1
20.32	10.8	16.1	10.7
15.8	10.47	11.1	10.3
13.41	7.9	11.7	4.1
8.67	3.55	9.17	3.7
8–9 am serum cortisol values in group 2 (PAI)			
4.57	6.57	ND	ND
0.92	0.93	1	1
13.9	11.3	11.8	10.5
4.55	3.39	5.53	1.7
0.5	0.4	1	0.4
0.5	0.4	1	1
10.18	7.96	9.76	7.5
0.92	0.75	1	0.6
8.69	6.45	6.15	6.1
5.2	4.14	5.27	3.8
1.92	2.86	2.42	2.5
Serum cortisol values (post ONDST) in group 3 (CS)			
20.8	17.2	20	14.9
71.8	60	ND	58.2
5.47	5.77	4.13	5.8
64.27	44	40	34
22.15	18.35	ND	ND
34.13	34.37	41.6	26.3
Serum cortisol values (post ONDST) in group 4 (AI)			
4.57	4.37	4.64	3.7
1.7	1.54	1.39	1.5
5.88	8.79	7.25	6.9
0.92	1.01	1	1.1
1.69	1.59	1.99	1.6
ND	0.65	ND	0.5
0.73	0.97	1	0.7
2.3	2.54	2.07	ND
8–9 am serum cortisol values in group 5 (HS)			
12.6	10.7	9.15	8.2
14.3	13.88	12.2	11.7
17	14.73	12.5	13.5
13.8	13.88	11.9	11.2
15.7	12.86	14.9	9.2
31.6	32.6	23.1	25
15	15.53	12.9	11.9
14.9	21.96	16.3	17.7
19.1	13.37	15	10.9
8.3	8.02	17.8	13.5

AA=Abbott Architect, AI=adrenal incidentaloma, CAH=congenital adrenal hyperplasia, CS=Cushing's syndrome, HS=healthy subjects, mcg/dL=microgram per decilitre, ND=not determined, ONSDT=overnight dexamethasone suppression test, PAI=primary adrenal insufficiency, RCe411=Roche Cobas e411, SAC XP=Siemens Advia Centaur XP, SI 1000=Siemens Immulite 1000

dL. On the other hand, serum cortisol was less than 5 µg/dL in six samples (50%) when measured with RC e411.The diagnosis of CAH, in which hypocortisolism is almost universal, might be missed if cortisol is measured in the SAC XP platform.

Corticosterone cross-reactivity with cortisol has been reported to be 2.6%, 2.48%, and 0.9% in SAC XP, RC e411, and AA analyzers, respectively, when spiked at a concentration of 1000 µg/dL in the test sample. However, it was 0.92% in the SI 1000 analyzer at a concentration of 400 µg/dL. Such interferences might be relevant in patients with CAH due to CYP17A1 defect.

A study conducted in treatment naive patients with CS, found that cut-offs for the ONDST-cortisol varied among IAs. Abbott Architect, Roche E170, Beckman Access, and Siemens Centaur platforms reported mean biases of − 19.5 nmol/L (−0.71 µg/dL), 15.2 nmol/L (0.55 µg/dL), 0.3 nmol/L (0.01 µg/dL), and − 3.9 nmol/L (−0.14 µg/dL), respectively, compared to the gold standard LC-MS/MS.[18]

In the present study, in contrast to CAH, excellent agreements between different analyzers were found in group II (PAI other than CAH), group III (endogenous CS), and group IV (AI). Cortisol values correlated well across all analyzers in healthy participants (group V). Circulatory concentrations of intermediates of steroid metabolism, with potential for significant cross-reactivity with cortisol, are much lower in these conditions compared to CAH. When cortisol concentrations, measured by SAC XP, were compared with LC-MS/MS in an earlier study, the two methods showed excellent agreement in healthy participants and patients with CS (not on metyrapone treatment). However, a positive bias was observed in SAC XP compared to LC-MS/MS in metyrapone-treated CS due to interference by 11DOC.[19]

Our study encompassed patients with various adrenal disorders, acknowledging previous research suggesting that cortisol IAs may demonstrate interference, especially in certain platforms, particularly in metyrapone-treated patients. However, the specific interference encountered in different CLIA-based analyzers has not been systematically investigated in patients with CAH and AI. Notably, our findings indicate that in patients with CAH due to 21OHD and 11β–OHD, the measured serum cortisol concentrations can be misleading, particularly when assessed by SAC XP.

While our study has provided valuable insights, it is crucial to recognize its limitations. First and foremost, the unavailability

of LC-MS/MS restricted our ability to compare different IAs with the gold standard method. Additionally, the diagnosis of CAH relied solely on clinical findings and appropriate investigations, lacking confirmation through genetic analysis. The relatively small number of participants in each group and the missing values in some analyzers are also noteworthy limitations. Specifically, some cortisol values in the CAH group were unavailable in the Abbott Architect Plus and Immulite 1000 analyzers due to reagent shortages during the ongoing COVID-19 pandemic. In addition, as the sample size in each group is small, summary values may not be truly representative. Furthermore, we acknowledge that not all steroid precursors known to interfere with cortisol immunoassay were measured. Our assessment was confined to 17OHP, and even this measurement was restricted to the CAH group. Consequently, a multivariate analysis demonstrating the impact of various steroid precursor concentrations on measured cortisol concentration could not be performed. It is essential to consider these limitations in interpreting the findings of our study.

Conclusion

Most of the IAs, commonly used for cortisol estimation in routine clinical practice, are susceptible to interference due to cross-reactivity with different steroid intermediates. Such interference is most relevant in patients with CAH, where the diagnosis of hypocortisolism may be missed if serum cortisol is measured by the SAC XP analyzer. In addition, 11β–OHD may be misdiagnosed as PGR. RC e411, SI 1000, and AA analyzers perform better in these patients. We conclude that cortisol should not be measured by SAC XP in individuals with suspected CAH. Cortisol measurement by all available platforms does not make any clinically significant differences in other forms of PAI, patients with endogenous CS, or AI. Knowledge of such interference in different IAs is important for clinicians to avoid diagnostic and therapeutic misadventures.

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Authors' contribution

Conceptualization: Partha Pratim Chakraborty. Data collection and analysis: Neeti Agrawal, Partha Pratim Chakraborty, Rana Bhattacharjee, Avivar Awasthi. Management of patients: Neeti Agrawal, Anirban Sinha, Partha Pratim Chakraborty, Rana Bhattacharjee, Avivar Awasthi, Animesh Maiti. Statistical analysis: Rana Bhattacharjee. Writing - original draft preparation: Neeti Agrawal, Partha Pratim Chakraborty. Writing - review and editing: Rana Bhattacharjee. Supervision: Anirban Sinha, Animesh Maiti.

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Conflicts of interest

There are no conflicts of interest.

Data availability

Data are available upon reasonable request by contacting the corresponding author.

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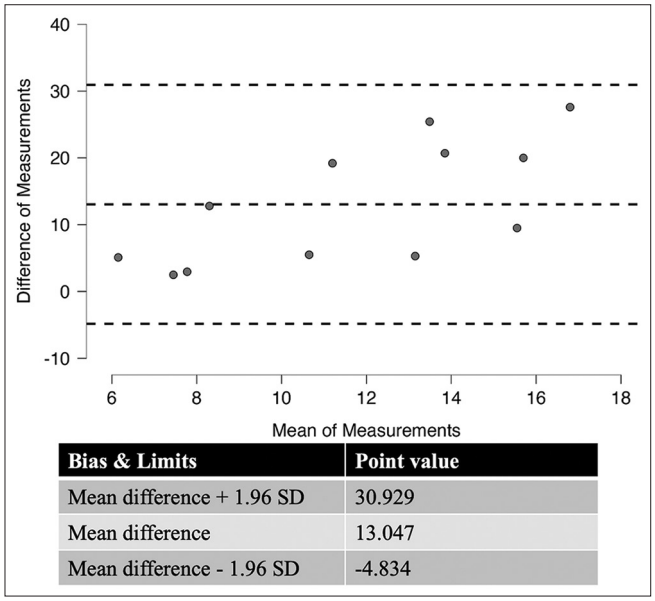
Supplementary File-Abbreviations and Bland-Altman Plots

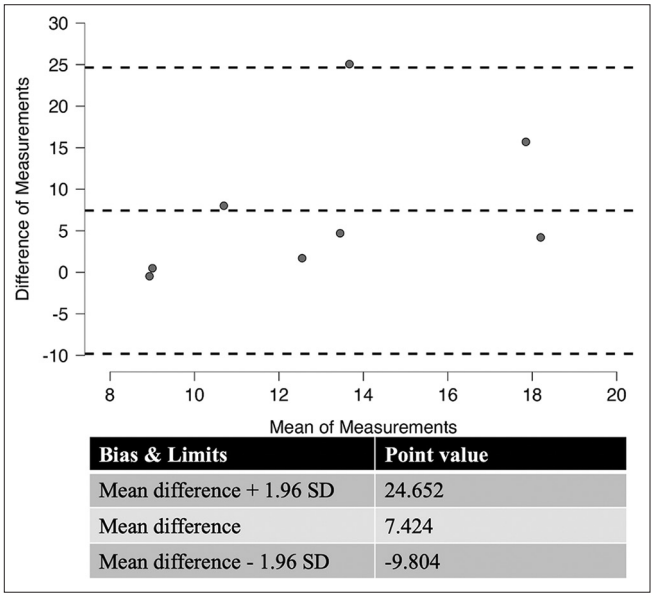
Abbreviations

AA	Abbott Architect
ACTH	Adrenocorticotrophin hormone
AI	Adrenal incidentaloma
AM	Ante-Meridiem
CAH	Congenital adrenal hyperplasia
CBG	Cortisol binding globulin
CI	Confidence interval
CLIA	Chemiluminescent immunoassay
CS	Cushing's Syndrome
11-DOC	11-Deoxycortisol
21-DOC	21-Deoxycortisol
FGD.	Familial Glucocorticoid Deficiency
ICC	Intraclass correlation coefficient
LCMS	Liquid chromatography mass spectrometry
µg/dl	Microgram per decilitre
NNT	Nicotinamide Nucleotide Transhydrogenase
17(OH)P	17α-hydroxyprogesterone
ONDST	Overnight dexamethasone suppression test
PAI	Primary adrenal insufficiency
PGR	Primary Glucocorticoid Resistance
pg/ml	Picogram per decilitre
RCe411	Roche Cobas e411
SAC XP	Siemens Advia Centaur XP
SARS-Cov2	Severe acute respiratory syndrome coronavirus 2
SI 1000	Siemens Immulite 1000
SPSS	Statistical Product and Service Solutions



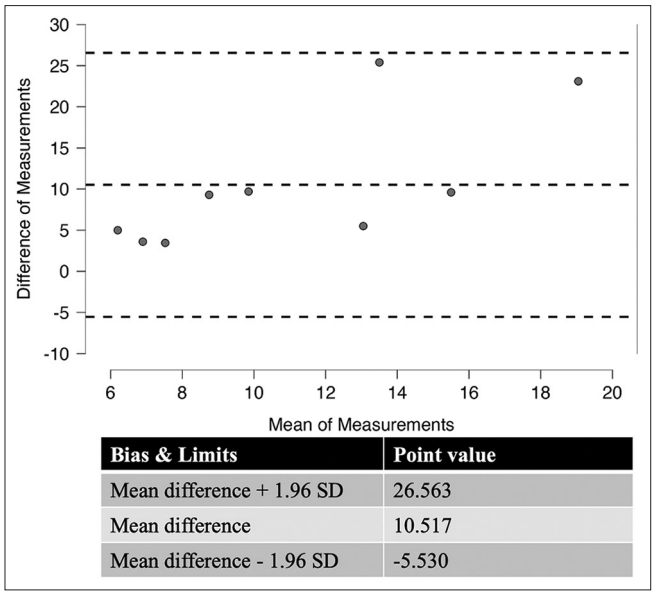
Bias & Limits	Point value
Mean difference + 1.96 SD	30.929
Mean difference	13.047
Mean difference - 1.96 SD	-4.834

Supplementary Figure 1: Bland-Altman plot and table and showing magnitude of agreement between SAC XP and RC e411 in patients with CAH. The dotted line in the middle of the Bland-Altman plot indicates the mean difference of 13.047 and dotted lines above and below indicate the 95% limits of agreement



Bias & Limits	Point value
Mean difference + 1.96 SD	24.652
Mean difference	7.424
Mean difference - 1.96 SD	-9.804

Supplementary Figure 2: Bland-Altman plot and table and showing magnitude of agreement between SAC XP and SI 1000 in patients with CAH. The dotted line in the middle of the Bland-Altman plot indicates the mean difference of 7.424 and dotted lines above and below indicate the 95% limits of agreement



Bias & Limits	Point value
Mean difference + 1.96 SD	26.563
Mean difference	10.517
Mean difference - 1.96 SD	-5.530

Supplementary Figure 3: Bland-Altman plot and table and showing magnitude of agreement between SAC XP and AA in patients with CAH. The dotted line in the middle of the Bland-Altman plot indicates the mean difference of 10.517 and dotted lines above and below indicate the 95% limits of agreement