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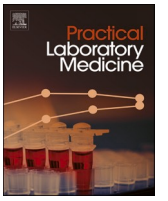

# Comparative analysis of salivary cortisol measurements using different assay methods in relation to serum-free cortisol measurement

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ABSTRACT

*Objectives:* Salivary cortisol reflects the biologically active form of serum cortisol, offering a noninvasive evaluation method for the diurnal rhythm of the hypothalamic-pituitary-adrenal (HPA) axis. While liquid chromatography-tandem mass spectrometry (LC-MS/MS) is known for its specificity, immunoassays (IA) are commonly used because of their simplicity. This study aimed to assess the performance of salivary cortisol measurement using both IA and LC-MS/MS in comparison to serum-free cortisol measurement.

*Methods:* Assay results for 188 saliva and 94 serum samples from 47 participants were analyzed. Salivary samples collected at different time points were analyzed using IA and LC-MS/MS. Serum samples were analyzed for cortisol, cortisol-binding globulin, and free cortisol. The statistical analyses included correlations and method comparisons.

*Results:* The diurnal salivary cortisol profiles exhibited a comparable circadian rhythm pattern; however, the concentrations measured using IA were consistently higher than those measured using LC-MS/MS. The correlation analysis revealed robust associations among salivary cortisol (IA), salivary cortisol (LC-MS/MS), and serum-free cortisol levels (LC-MS/MS). However, the method comparison revealed a systematic bias between IA and LC-MS/MS in salivary cortisol measurement.

*Conclusions:* This study contributes to the ongoing debate on assay techniques by affirming the suitability of IA and LC-MS/MS for salivary cortisol measurement to assess dynamic changes in HPA axis activity. The identified systematic bias emphasizes the importance of selecting methods based on specific research or clinical requirements.

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## 1. Introduction

**Abbreviations**

| | |
|---|---|
| 11β-HSD2 | 11β-hydroxysteroid dehydrogenase type 2 |
| A0 | 0 min |
| A30 | 30 min |
| A60 | 60 min |
| N | 9 p.m. |
| CMIA | chemiluminescent microparticle immunoassay |
| CV | coefficient of variance |
| ELISA | enzyme-linked immunoassay |
| HPLC | high-performance liquid chromatography |
| HPA | hypothalamic-pituitary-adrenal |
| IA | immunoassays |
| IS | internal standard |
| IQR | interquartile range |
| LOD | limit of detection |
| LOQ | limit of quantification |
| LC-MS/MS | liquid chromatography-tandem mass spectrometry |
| MS | mass spectrometry |
| Sal $C_{MS}$ | salivary cortisol analyzed using mass spectrometry |
| Sal $C_{IA}$ | salivary cortisol analyzed using immunoassay |
| Sal $CE_{MS}$ | salivary cortisone analyzed using mass spectrometry |
| Sr $C_{CMIA}$ | serum cortisol analyzed using chemiluminescent microparticle immunoassay |
| Sr CBG | serum cortisol-binding globulin |
| Sr $fC_{MS}$ | serum free cortisol analyzed using mass spectrometry |
| Sr FCI | serum free cortisol index |

Salivary cortisol reliably reflects the biologically active form of serum cortisol [1]. Furthermore, it offers the benefit of noninvasive sample collection while mirroring the diurnal rhythm of serum-free cortisol.

The diurnal salivary cortisol profiles play a pivotal role in understanding deviations in absolute hormone levels and circadian rhythms, which have been associated with various physiological and psychiatric disorders [2,3]. Therefore, accurate measurements of varying hormone levels hold the utmost importance.

Mass spectrometry (MS) is being increasingly recognized as the gold standard for hormonal measurement owing to its high specificity compared to immunoassays (IA). However, salivary cortisol is commonly measured using IA because of its simplicity and cost efficiency [4]. This study aimed to compare liquid chromatography-tandem mass spectrometry (LC-MS/MS) and IA in the measurement of salivary cortisol, particularly in relation to serum-free cortisol measurement using LC-MS/MS.

## 2. Materials and methods

### 2.1. Participants

In this study, 188 saliva and 94 blood samples were collected from 47 participants between February and September 2022 from the outpatient department at Gangnam Severance Hospital, Seoul, Korea. The collection was done as a part of an exploratory clinical trial for the Mids. NAVI software program [5]. The participants included 12 healthy controls and 35 individuals with major depressive disorders (6 with mild depression, 22 with moderate depression, and 7 with severe depression). The median age of the participants was 28.0 (range 19.0–50.0) years, with 28 (59.6 %) female individuals. The median body mass index was 22.9 (range 16.6–43.8) $kg/m^2$. None of the participants used any medications or dietary supplements that could affect the HPA axis. The study was approved by the Institutional Review Boards of Severance Hospital (No-3-2012-0085) and Gangnam Severance Hospital (No.3-2021-0440). Written informed consent was obtained from all individual patients included in the study.

### 2.2. Specimen collection

Salivary samples were collected using the passive drool method [6], where participants were required to pool saliva in their mouths for 2 min and then expel it into a collection vial. Saliva was collected at 9 p.m. (N), and then at 0 (A0), 30 (A30), and 60 min (A60) after awakening. The participants provided 2 mL of saliva into designated tubes at each time point. Immediately after collection, the saliva samples were stored at −20 °C until analysis.

Additionally, two separate venipunctures were conducted: one at time-point N and one at A60. Peripheral venous blood was drawn into sterile vacuum collection tubes, allowed to clot, and centrifuged at 1650×*g* for 15 min. The resulting serum was aliquoted and stored at −80 °C until analysis.

### 2.3. Salivary cortisol measurement using IA

After thawing, the saliva samples were centrifuged at 1650×*g* for 15 min. Salivary cortisol IA (Sal $C_{IA}$) was performed using an enzyme-linked immunoassay (ELISA) kit, using the Triturus DG-53 ELISA processor (Grifols Diagnostic, Barcelona, Spain), and a Cortisol Saliva ELISA kit (IBL International GmBH, Hamburg, Germany: Lot No. RE52611). The Sal $C_{IA}$ concentrations showed consistent linearity over a range of 0.15–71.58 nmol/L with a correlation coefficient of 0.999. The intraassay precision had coefficient of variances (CVs) of 3.0 %–4.7 %, the inter-assay precision had CVs of 2.6 %–5.9 % across concentrations of 1.5–32.2 nmol/L, and the limit of quantification (LOQ) was 0.138 nmol/L.

### 2.4. Salivary cortisol and cortisone assays using LC-MS/MS

Analyses of salivary cortisol (Sal $C_{MS}$) and cortisone (Sal $CE_{MS}$) using LC-MS/MS were performed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, Canada) coupled with an Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA) liquid chromatography system. To analyze Sal $C_{MS}$ and Sal $CE_{MS}$ simultaneously, cortisol-$d_4$ dissolved in methanol to obtain a stock solution of 36.7 nmol/L was used as the internal standard (IS). A 20 μL aliquot of IS was spiked into 500 μL of the saliva sample; then, 2 mL of ethyl acetate was added to the mixture. The mixture was vortex-mixed for 30 s and then centrifuged at 1650×*g* for 15 min. The mixture separated into the aqueous layer and organic layer was placed in a deep freezer at −80 °C for 15 min, and the supernatant was evaporated to dryness at 40 °C under nitrogen. The resulting pellet was dissolved in 100 μL of methanol and then 20 μL of the dissolved solution was injected into the LC-MS/MS system. The Sal $C_{MS}$ concentrations showed consistent linearity over a range of 0.04–7.25 nmol/L with a correlation coefficient of 0.999. The intraassay and inter-assay precisions of Sal $C_{MS}$ had CVs of 0.6 %–2.8 % and 0.3 %–3.4 %, respectively. The LOQ of Sal $C_{MS}$ was 0.036 nmol/L. The cortisol: cortisone ratio for individual samples was calculated by dividing the Sal $C_{MS}$ by the Sal $CE_{MS}$.

### 2.5. Analyses of serum cortisol and cortisol-binding globulin

After thawing, the serum samples were centrifuged at 1650×*g* for 15 min. Serum cortisol concentrations (Sr $C_{CMIA}$) were measured using a chemiluminescent microparticle immunoassay (CMIA) on an Alinity i automated analyzer (Abbott Laboratories, Chicago, IL, USA). The intraassay and inter-assay CVs of Sr $C_{CMIA}$ were <5.1 %; the limit of detection (LOD) was 19.3 nmol/L. The LOQ was 27.6 nmol/L. Serum cortisol-binding globulin (Sr CBG) was measured using ELISA with a human corticosteroid-binding globulin ELISA kit (BioVnedor R&D, Brno, Czech Republic). The intraassay and inter-assay CVs were less than 5.3 % and 4.3 %, respectively, according to the manufacturer's information. The serum free cortisol index (Sr FCI) was calculated as a surrogate marker for free cortisol by dividing the Sr $C_{CMIA}$ (nmol/L) by the Sr CBG (nmol/L) [7].

### 2.6. Serum-free cortisol assay using LC-MS/MS

The serum was filtered using 10,000-Da-molecular-weight-cutoff Amicon Ultra-0.5 mL centrifugal filters (Merck Life Science Limited, Wicklow, Ireland). Subsequently, 300 μL of ultrafiltered serum was used to analyze serum-free cortisol (Sr $fC_{MS}$). LC-MS/MS extraction was performed according to the manufacturer's instructions using an LC-MS/MS high–performance liquid chromatography

**Table 1**
Comparison of salivary and serum cortisol profiles by method and sampling time.

| Analytes and methods (n = 47) | Time | | | |
|---|---|---|---|---|
| | A0 | A30 | A60 | N |
| | Mean (Median, q1–q3) | Mean (Median, q1–q3) | Mean (Median, q1–q3) | Mean (Median, q1–q3) |
| Sal $C_{IA}$ (nmol/L) | 2.7[a] (3.0, 1.6–5.5)[a] | 9.5 (7.3, 5.1–13.9) | 6.1[a] (7.8, 3.3–11.3)[a] | 1.8[a] (2.0, 0.8–3.6)[a] |
| Sal $C_{MS}$ (nmol/L) | 1.0[a] (1.4, 0.5–2.5)[a] | 4.8 (3.9, 2.2–7.1) | 2.4[a](3.2, 1.6–5.7)[a] | 0.6[a] (0.7, 0.2–1.7)[a] |
| Sal $CE_{MS}$ (nmol/L) | 10.3[a] (11.1, 6.7–18.7)[a] | 23.1 (22.5, 17.0–30.0) | 19.6[a](22.6, 16.4–27.6)[a] | 8.5[a](9.1, 5.1–13.7)[a] |
| Sal $C_{MS}$/Sal $CE_{MS}$ ratio | 0.12 (0.13, 0.08–0.15) | 0.18 (0.19, 0.12–0.24) | 0.15 (0.14, 0.10–0.21) | 0.09 (0.08, 0.04–0.14) |
| Sr $fC_{MS}$ (nmol/L) | – | – | 8.8[a] (10.4, 6.4–16.2)[a] | 3.2[a] (3.0, 1.4–7.0)[a] |
| Sr $C_{CMIA}$ (nmol/L) | – | – | 280.0 (295.3, 204.0–353.3) | 125.8[a](138.0, 59.3–256.6)[a] |
| Sr $fC_{MS}$/Sr $C_{CMIA}$ ratio | – | – | 0.04[a] (0.04, 0.03–0.05)[a] | 0.03[a](0.02, 0.02–0.03)[a] |
| Sr CBG (μnmol/L) | – | – | 0.47[a](0.45, 0.42–0.50)[a] | 0.47[a](0.45, 0.42–0.52)[a] |
| Sr FCI | – | – | 0.61 (0.68, 0.43–0.78) | 0.27[a] (0.30, 0.15–0.48)[a] |

[a] Back transformed after logarithmic transformation. A0, A30, and A60 represent immediate, and 30 and 60 min after awakening, respectively; N, 9 p.m.; Sal $C_{IA}$: salivary cortisol analyzed using immunoassay; Sal $C_{MS}$: salivary cortisol analyzed using mass spectrometry; Sal $CE_{MS}$: salivary cortisone analyzed using mass spectrometry; Sr $fC_{MS}$: serum free cortisol analyzed using mass spectrometry; Sr $C_{CMIA}$: serum cortisol analyzed using chemiluminescent immunoassay; Sr CBG: serum cortisol binding globulin; Sr FCI: serum free cortisol index.

(HPLC) system (Shimadzu, Kyoto, Japan) coupled to Triple Quad 6500+ (SCIEX, Framingham, USA). The calibration curves of Sr $fC_{MS}$ were linear over the free cortisol calibration range (0.28–1380 nmol/L), with a correlation coefficient of 0.999. The LOQ of the free cortisol assay was 0.28 nmol/L with average intraassay and intra-assay CVs of 1.0%–6.0 % and 8.7%–11.9 %, respectively. The free cortisol: cortisol ratio in individual samples was calculated by dividing the Sr $fC_{MS}$ by the Sr $C_{CMIA}$, aiming to investigate the relative proportion of free cortisol to cortisol in the blood.

### 2.7. Statistical analyses

The data for the groups are presented as medians and interquartile ranges (IQR) when appropriate. Spearman's correlation analysis was used to estimate the correlations between the analyses. Analytical method comparison was performed using Passing–Bablok regression analysis, and differences between measurement methods were illustrated using Bland–Altman plots. Data analysis was performed using the MedCalc Statistical Software version 22.014 (MedCalc Software, Ostend, Belgium), and statistical significance was set at $p < 0.05$.

## 3. Results

### 3.1. Diurnal salivary cortisol profiles

Table 1 and Fig. 1 present the concentrations of diurnal salivary cortisol profiles by method and sampling time across 14 h in a day, showing comparable circadian rhythm patterns in both Sal $C_{IA}$ and Sal $C_{MS}$. The mean Sal $C_{MS}$/Sal $CE_{MS}$ ratios at different time points were as follows; 0.12 (A0), 0.18 (A30), 0.15 (A60), and 0.09 (N). The mean Sr $fC_{MS}$/Sr $C_{CMIA}$ ratios were 0.04 (A60) and 0.03 (N); the mean values of Sr FCI were 0.61 (A60) and 0.27 (N) (Table 1). While a comparable circadian rhythm pattern was observed in both Sal $C_{IA}$ and Sal $C_{MS}$ for each case, the concentrations of Sal $C_{IA}$ were consistently higher than those of Sal $C_{MS}$ (Fig. 2).

### 3.2. Correlation between serum and saliva measurements

When assessing the correlation between serum and saliva measurements, the concentrations of Sal $C_{IA}$ strongly correlated with those of Sal $C_{MS}$ and Sal $CE_{MS}$ (r = 0.966 and 0.952, respectively). Additionally, Sr $fC_{MS}$ strongly correlated with Sr $C_{CMIA}$, Sal $C_{IA}$, Sal $C_{MS}$, and Sr FCI (r = 0.977, 0.858, 0.868, and 0.960, respectively) (Fig. 3).

### 3.3. Comparison of salivary cortisol measurements using IA and LC-MS/MS in relation to serum-free cortisol measurement

The results of the method comparison of Sal $C_{IA}$ and Sal $C_{MS}$ with Sr $fC_{MS}$ are shown in Fig. 4. Despite both methods showing a strong correlation with Sr $fC_{MS}$, they showed a negative bias compared to Sr $fC_{MS}$ (mean difference; Sr $fC_{MS}$ vs. Sal $C_{IA}$: 2.2 nmol/L, Sr$fC_{MS}$ vs. Sal $C_{MS}$: 5.4 nmol/L). Furthermore, Sal $C_{IA}$ showed a positive bias compared to Sal $C_{MS}$ (mean difference: 3.2 nmol/L), despite both methods demonstrating a strong correlation (r = 0.966, $p < 0.0001$).

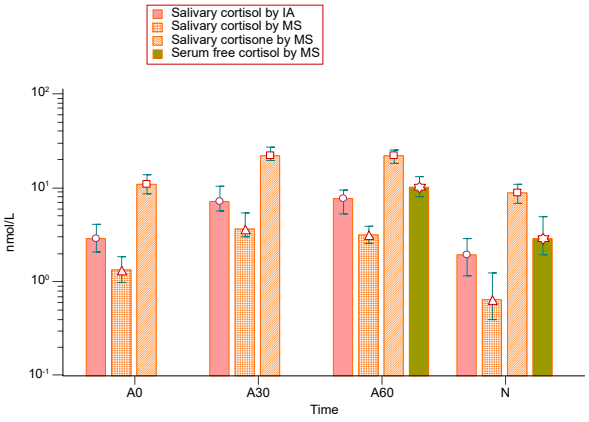


**Fig. 1.** Comparison of salivary and serum analytes by analytical method and sampling time (n = 47). Serum-free cortisol was analyzed using samples collected at time points A60 and N only. Solid pink: salivary cortisol (IA); orange diamonds: salivary cortisol (MS); orange stripe: salivary cortisone (MS); solid green: serum-free cortisol (MS). A0, A30, and A60 present samples collected immediately, and 30, and 60 min after awakening, respectively; N: samples collected at 9 p.m. IA: immunoassay; MS: mass spectrometry. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

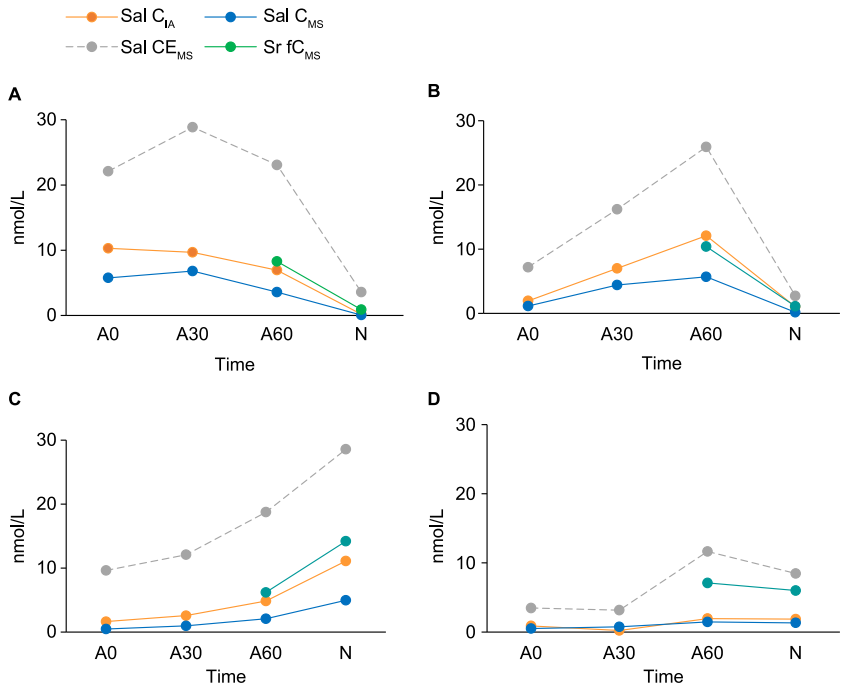


**Fig. 2.** Cases of cortisol profile. (A) Normal cortisol awakening response; (B) late increase of cortisol awakening; (C) reversed cortisol awakening; (D) overall decreased cortisol awakening response. Sal $C_{IA}$: salivary cortisol analyzed using immunoassay; Sal $C_{MS}$: salivary cortisol analyzed using mass spectrometry; Sal $CE_{MS}$: salivary cortisone analyzed using mass spectrometry; Sr $fC_{MS}$: serum free cortisol analyzed using mass spectrometry.

| | Sal $C_{MS}$ | Sr $fC_{MS}$ | Sr FCI | Sal $C_{IA}$ | Sr $C_{CMIA}$ |
|---|---|---|---|---|---|
| Sr $fC_{MS}$ | 0.868 P <0.0001 | | | | |
| Sr FCI | 0.879 P <0.0001 | 0.960 P <0.0001 | | | |
| Sal $C_{IA}$ | 0.966 P <0.0001 | 0.858 P <0.0001 | 0.872 P <0.0001 | | |
| Sr $C_{CMIA}$ | 0.849 P <0.0001 | 0.977 P <0.0001 | 0.932 P <0.0001 | 0.839 P <0.0001 | |
| Sal $CE_{MS}$ | 0.956 P <0.0001 | 0.832 P <0.0001 | 0.849 P <0.0001 | 0.952 P <0.0001 | 0.799 P <0.0001 |

**Fig. 3.** Correlogram of salivary and serum analytes. Spearman rank correlation coefficients were estimated between 94 matched results of salivary and serum analytes. Sr $fC_{MS}$: serum free cortisol analyzed using mass spectrometry; Sr FCI: serum free cortisol index; Sal $C_{IA}$: salivary cortisol analyzed using immunoassay; Sr $C_{CMIA}$: serum cortisol analyzed using chemiluminescent immunoassay; Sal $CE_{MS}$: salivary cortisone analyzed using mass spectrometry; Sal $C_{MS}$: salivary cortisol analyzed using mass spectrometry.

## 4. Discussion

Blood cortisol is normally bound by CBG and albumin, preventing glucocorticoids from penetrating the membranes of target cells [1]. Despite CBG playing a critical role in regulating the bioavailability and metabolic clearance of glucocorticoids, only 3%–5% of total blood cortisol exists in its bioactive form as unbound free cortisol [8]. The mean results of the ratio of serum-free cortisol to serum cortisol were 0.04 and 0.03 (A60 and N) in this study, consistent with previous study results [8,9].

In the serum, the cortisol level considerably exceeds the cortisone level, at a ratio of approximately 4:1 [10]. Free cortisol diffuses through acinar cells into the salivary gland, where it is rapidly converted to cortisone by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), as salivary glands have high levels of 11β-HSD2 [11]. Therefore, in the saliva, the cortisol: cortisone ratio is reversed and is in the range of 0.13–0.22 throughout the day [12]. The findings of our study are consistent with these results, as the mean cortisol: cortisone ratios ranged between 0.09 and 0.18 (Table 1). The possible reason for higher cortisol: cortisone ratios in the morning than in the night might indicate saturation of the salivary gland 11β-HSD2 due to cortisol surge in the morning [13]. Salivary cortisone has been identified as a superior marker of serum cortisol compared to salivary cortisol, especially in situations where serum cortisol is low during hydrocortisone therapy, or when blood contamination in saliva samples may lead to misleading high cortisol concentrations [11].

Previous studies [14–16] have indicated the IA measurements of salivary cortisol are approximately 2–2.5 times higher than those obtained through MS; our results are consistent with this. MS is preferred over IA in cortisol analysis because of the potential

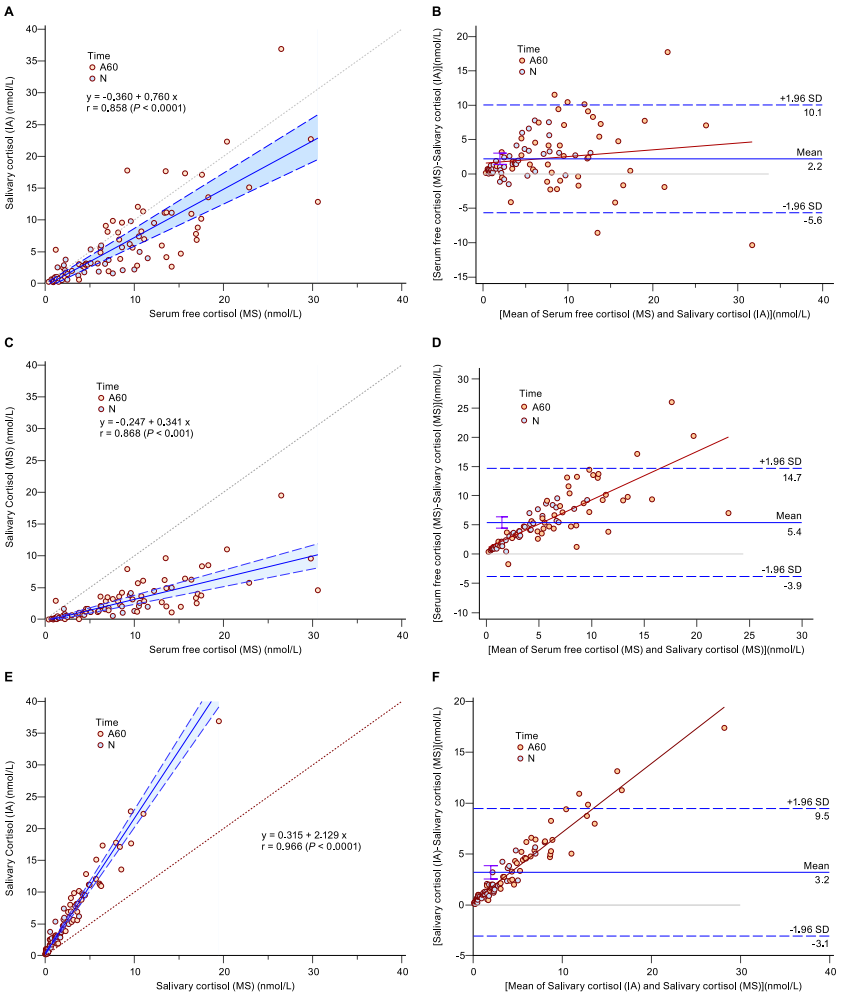


**Fig. 4.** Comparative analysis of salivary cortisol measurements using immunoassay and LC-MS/MS in relation to serum-free cortisol measurement. (A) Passing–Bablok regression plot of Sal $C_{IA}$ and Sr $fC_{MS}$; $y = -0.360 + 0.760x$ ($r = 0.858$, $p < 0.0001$). (B) Bland–Altman plot for the method comparison between Sal $C_{IA}$ and Sr $fC_{MS}$. The y-axis corresponds to the difference in Sr $fC_{MS}$ and Sal $C_{IA}$ values (mean difference: 2.2 nmol/L, 95 % confidence interval [CI]: 1.4–3.0 nmol/L). (C) Passing–Bablok regression plot of Sal $C_{MS}$ and Sr $fC_{MS}$; $y = -0.247 + 0.341x$ ($r = 0.868$, $p < 0.0001$). (D) Bland–Altman plot for the method comparison between Sal $C_{MS}$ and Sr $fC_{MS}$. The y-axis corresponds to the difference in serum Sr $fC_{MS}$ and Sal $C_{MS}$ values (mean difference: 5.4 nmol/L, 95 % CI: 4.4–6.4 nmol/L). (E) Passing–Bablok regression plot of Sal $C_{IA}$ and Sal $C_{MS}$; $y = 0.315 + 2.129x$ ($r = 0.966$, $p < 0.0001$). (F) Bland–Altman plot for the method comparison between Sal $C_{IA}$ and Sal $C_{MS}$. The y-axis corresponds to the difference in Sal $C_{IA}$ and Sal $C_{MS}$ values (mean difference: 3.2 nmol/L, 95 % CI: 2.6–3.9 nmol/L).

cross-reactivity of cortisol metabolites in IA. According to the manufacturer's information, several compounds, including prednisolone (16.64 %), 11-deoxycortisol (8.53 %), cortisone (2.55 %), 17-α-hydroxyprogesterone (1.29 %), and prednisone (1.23 %), can cross-react with cortisol saliva ELISA assay (IBL). Among these, cortisone appears to be a major interfering compound in IA, given its notably high concentration in saliva. Poor standardization, partly due to differences in antibody specificity and partly the lack of single reference material, is another factor contributing to the variability of cortisol immunoassays [17]. Bäcklund et al. compared six different analytical methods for salivary cortisol in diagnosing Cushing's syndrome (CS), reporting similar absolute values among three LC-MS/MS methods but different values among three IAs [18]. Some studies have suggested that IAs are more sensitive to CS and better reflect total glucocorticoid overload because of cross-reactivity with cortisol metabolites [19,20], whereas others have demonstrated no clear difference in diagnostic accuracy between IAs and MS methods [21]. Meanwhile, it is noteworthy that the difference between serum-free cortisol and salivary cortisol concentrations was more pronounced in LC-MS/MS than in IA in this study. Recently, the choice of assay technique has been the focus of vigorous debate in steroid analysis, including salivary cortisol. Experts in the field of clinical chemistry advocate for MS and simultaneously recognize that the well-validated IA is more than adequate to assist in clinical decision-making [14].

The primary objective of this study was to examine the correlation between serum-free cortisol (measured using LC-MS/MS) and salivary cortisol (measured using IA and LC-MS/MS). However, the study is subject to several limitations. First, the small number of participants constrained the ability to identify significant differences in salivary cortisol profiles between healthy controls and

individuals with major depressive disorders (data not shown). Second, serum-free cortisol levels were assessed at only two-time points (N and A60) instead of four. Despite these limitations, we explored this relationship across a diverse range of cortisol values, particularly in conditions where cortisol concentrations rapidly change, transitioning from nadir to peak levels throughout the day.

Previously published data comparing IA and MS as salivary cortisol analysis methods have been limited to different methods of salivary cortisol comparisons, and these studies did not include data on paired salivary and serum samples [14–16]. In this regard, this study offers a direct comparison of salivary cortisol with serum-free cortisol, using LC-MS/MS as the reference method, in samples obtained simultaneously.

Importantly, this study demonstrated that both IA and LC-MS/MS methodologies are suitable for assessing dynamic changes in HPA axis activity, especially during periods when cortisol concentration changes rapidly. However, the observed systematic bias between IA and LC-MS/MS emphasizes the importance of method selection based on specific research and clinical requirements.

## 5. Conclusions

In conclusion, this study provides valuable insights into the measurement of salivary cortisol using the IA and LC-MS/MS methods, with a comprehensive comparison against serum-free cortisol measured by LC-MS/MS. The diurnal salivary cortisol profiles revealed a consistent circadian rhythm; however, the concentrations measured using IA were consistently higher than those measured using LC-MS/MS, demonstrating a systematic bias. This study would contribute to the ongoing debate on assay techniques, providing evidence that well-validated IA remains a valuable tool in clinical decision-making alongside the recognized specificity of LC-MS/MS.

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## Patient consent

Written informed consent was obtained from all individual patients included in the study.

## Ethical approval

This study was approved by the Institutional Review Boards of Severance Hospital (No-3-2012-0085) and Gangnam Severance Hospital (No.3-2021-0440).

## CRediT authorship contribution statement

**Anna Lee:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization, Data curation, Formal analysis, Methodology. **Sooah Jang:** Methodology, Writing – review & editing. **Sanghoo Lee:** Writing – original draft, Methodology. **Hyun-Kyung Park:** Writing – original draft, Methodology. **In-Young Kim:** Project administration, Methodology. **Ryunsup Ahn:** Methodology, Conceptualization. **Jeong-Ho Seok:** Conceptualization, Supervision. **Kyoung-Ryul Lee:** Conceptualization, Supervision.

## Declaration of competing interest

Jeong-Ho Seok is a professor at Yonsei University and the CEO of Minds. AI, Co., Ltd., which was established in Nov. 2019 as a research and development company for mental health services in Korea. Sooah Jang, In-Young Kim, and Ryunsup Ahn are employed by Minds. AI, Co., Ltd.

## Data availability

Data will be made available on request.

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