Original Article

A Comparison of 06:00 AM Versus 09:00 AM Serum Cortisol as a Basal Morning Cortisol in Guwahati, Assam: A Pilot Study

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Abstract

Introduction: Cortisol secretion is regulated by circadian rhythm, which is influenced by zeitgebers like light. In India, the entire country operates under a single time zone, Indian Standard Time, which may not align with the local sunrise timing across different regions. **Aims:** This study aimed to compare the basal serum cortisol levels between 06:00 AM and 09:00 AM in Guwahati, Assam, where sunrise occurs earlier compared with the western part of the country. A cross-sectional pilot study was conducted from December 2022 to June 2023 in a tertiary care hospital in Guwahati. **Methods:** Serum cortisol samples were collected at 06:00 AM and 09:00 AM from 25 healthy adult participants once in winter and again in summer. Descriptive statistics and paired Student's t-tests were used. **Results:** The mean serum cortisol levels at 06:00 AM in winter, summer and overall were 13.2, 13.4 and 13.3 µg/dL, respectively. At 09:00 AM, the mean serum cortisol levels in winter, summer and overall were 8.2, 7.7 and 8.0 µg/dL, respectively. Significant differences were observed between the 06:00 AM and 09:00 AM cortisol levels in both winter and summer (P <0.001). **Conclusion:** This study highlights the importance of considering the influence of earlier sunrise on circadian rhythm, cortisol secretion and sampling protocols. Recognising the impact of earlier sunrise on cortisol secretion and adapting sampling protocols accordingly to align with the local sunrise can provide a more accurate assessment of basal cortisol levels and help avoid potential misinterpretation and diagnostic challenges associated with low values.

Keywords: Basal cortisol, circadian rhythm, sampling time, zeitgeber

Introduction

Cortisol, a glucocorticoid hormone, is critical for normal body homeostasis. The pattern of cortisol secretion regulated by the hypothalamic-pituitary-adrenal (HPA) axis has been extensively studied. Normal secretory patterns include the circadian rhythm with maximum secretion occurring during the 6th, 7th and 8th hours of sleep and continuing through the first hour of wakefulness and minimum secretion occurring during 6-hour period of four hours before and 2 hours after the onset of sleep.[1] There is also an ultradian rhythm with pulses of cortisol secretion occurring hourly.[2] It is well established that the main driver of the circadian rhythm is the suprachiasmatic nucleus (SCN) of the hypothalamus, which responds to the ambient light-dark cycle.[3] Hence, changing daylight periods with changing seasons could potentially affect the timing of cortisol secretion. As per the recommended international guidelines, sampling for morning basal cortisol is performed between 08:00 AM. A basal cortisol concentration cut-off threshold of <140 nmol/L (5 µg/dL), measured in the morning (between 06:00 AM), indicates the possibility of adrenal insufficiency.[4]

The Republic of India spans two geographical time zones but uses a one time zone called India Standard Time (IST), which corresponds to Coordinated Universal Time (UTC) +05:30 throughout the entire country. Guwahati is situated at 26°10′20″ N and 91°44′45″ E. As sunrise in North East India, including Assam, is approximately 1.5 hours earlier than in Western India, occurring around 06:00 AM in December and January and 04:30 AM in June and July, interpreting a morning cortisol sample taken at 09:00 AM may be misleading, leading to an erroneous diagnosis of adrenal insufficiency or

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subjecting the patient to an unnecessary adrenocorticotropic hormone (ACTH) stimulation test. This is the first study conducted in Assam to compare the basal serum cortisol levels at 06:00 AM and at 09:00 AM, in both summer and winter.

Materials and Methods

This was a cross-sectional study conducted from December 2022 to June 2023 in a tertiary care hospital in Guwahati, Assam. The study group consisted of 25 participants with normal body mass index (BMI) without any comorbidities.

Inclusion criteria

Normal healthy adults aged between 18 and 40 years were included in the study.

Exclusion criteria

Patients with any endocrine disease, on steroid therapy, pregnant women, any chronic illness, night shift workers, patients with any psychiatric disorders or on psychiatric medications, patients on drugs known to affect cortisol metabolism or a family history of pituitary or adrenal tumours were excluded from the study.

Study procedure

A detailed clinical history and systemic examination of the study participants were recorded. After fulfilling the inclusion and exclusion criteria, samples for 06:00 AM serum cortisol and 09:00 AM serum cortisol were collected after an overnight fast of 10 hours in both January and June 2023 of the same 25 participants. Serum cortisol was measured using the Elecsys Cortisol II assay in the cobas e 411 analyzer, Roche Diagnostics. The inter- and intra-assay coefficient of variation was <4%.

The statistical analysis was performed using the latest version of Statistical Package for the Social Sciences (SPSS) software. The descriptive data were expressed as mean with standard deviation, percentage and counts. The paired Student's *t*-test was used to evaluate the difference between the two groups. A *P*-value less than 0.05 was considered significant. For this pilot study, a paired sample size of 25 was taken to assess the outcome of the study in both winter and summer.

Ethical aspects

The study was approved by Gauhati Medical College and Hospital Ethics Committee vide letter no. MC/190/2007/Pt-II/34 on 23 December 2022. Written informed consent was obtained for participation in the study and use of the participant data for research and educational purposes. The procedures follow the guidelines laid down in the Declaration of Helsinki 1964 and as revised later.

Results

The mean age of study participants was 27.6 ± 4.6 years, and 40% were women. The mean serum cortisol at 06:00 AM in winter, summer and overall was 13.2, 13.4 and 13.3 µg/dL, respectively, while serum cortisol at 09:00 AM in winter, summer and overall was 8.2, 7.7 and 8.0 µg/dL, respectively [Table 1]. The individual serum cortisol levels are depicted in Figure 1.

The mean winter serum cortisol level at 06:00 AM was significantly higher than at 09:00 AM, and the mean summer cortisol level at 06:00 AM was significantly higher than at 09:00 AM. The overall mean cortisol level, regardless of season, at 06:00 AM (13.3 µg/dL) was significantly higher than at 09:00 AM (8.0 µg/dL) with a *P*-value of <0.001 [Table 2].

The mean serum cortisol level at 06:00 AM was 13.2 ± 1.9 µg/dL during the winter and 13.4 ± 3.2 µg/dL during the summer. Similarly, at 09:00 AM, the mean serum cortisol level was 8.2 ± 2.8 µg/dL during the winter and 7.7 ± 2.9 µg/dL during the summer. However, there was no statistically significant difference in the mean serum cortisol levels between winter and summer at both 06:00 AM and 09:00 AM.

The mean fall in overall serum cortisol from 06:00 AM to 09:00 AM was 39.8% (5.3 µg/dL).

Discussion

Cortisol secretion is regulated by the circadian rhythm, which is influenced by external cues called zeitgebers, such as light-dark cycle,[5,6] awakening[7] and social cues.[8] Light, in particular,

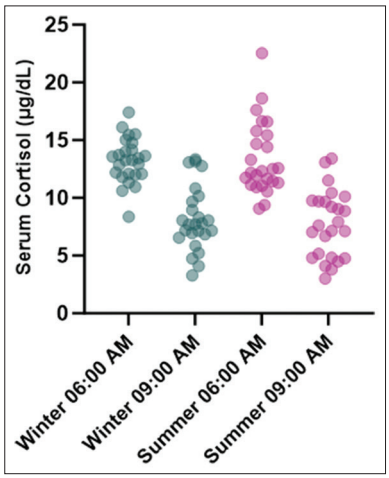

Figure 1: Serum cortisol concentrations at 06:00 AM and 09:00 AM

Table 1: Characteristics of the study participants (*n*=25)

Variables	Values
Age (years)	27.6±4.6
BMI (Kg/sqm)	20.8±1.3
Winter 06:00 AM serum cortisol (µg/dL)	13.2±1.9
Winter 09:00 AM serum cortisol (µg/dL)	8.2±2.8
Summer 06:00 AM serum cortisol (µg/dL)	13.4±3.2
Summer 09:00 AM serum cortisol (µg/dL)	7.7±2.9
Overall 06:00 AM serum cortisol (µg/dL)	13.3±2.6
Overall 09:00 AM serum cortisol (µg/dL)	8.0±2.8

BMI=Body mass index

Table 2: Comparison of serum cortisol sampled at 06:00 AM and 09:00 AM

Season	Serum cortisol sampling time	Mean (μg/dL)	Std. deviation	Mean difference	95% confidence interval of mean difference		*P*
					Lower	Upper	
Winter	6:00 AM	13.2	1.9	5	4.1	5.9	<0.001
	9:00 AM	8.2	2.8				
Summer	6:00 AM	13.4	3.2	5.7	4.5	6.9	<0.001
	9:00 AM	7.7	2.9				
Overall	6:00 AM	13.3	2.6	5.3	4.6	6.1	<0.001
	9:00 AM	8.0	2.8				

plays a crucial role in the entrainment of the circadian rhythm.[9] Normal secretory patterns include the circadian rhythm with maximum secretion occurring during the 6th, 7th and 8th hours of sleep and continuing through the first hour of wakefulness. This period represents the most intense adrenal cortical activity and accounts for approximately half of the total cortisol secreted over 24 hours. Minimum secretion occurs during the 6-hour period of 4 hours before and 2 hours after the onset of sleep.[1] In the context of India, where the entire country operates under a single time zone, that is IST, it is essential to recognise the potential variations in cortisol secretion due to differences in sunrise timing and light exposure across different regions.

In this study, we assessed the morning basal cortisol at two different time points, namely IST 06:00 AM and 09:00 AM in relation to sunrise in Guwahati, Assam, in normal subjects. It is important to note that Guwahati, being located in the eastern part of India, experiences an earlier sunrise compared with other regions of the nation. Serum cortisol at 09:00 AM was significantly less when compared to 06:00 AM. This observation was consistent across both the summer and winter seasons, indicating that an earlier sunrise in Guwahati led to an earlier acrophase of cortisol secretion and started falling after that. Also, it was found that there was a drop in serum cortisol by 38.9% from 06:00 AM to 09:00 AM. In other terms for each hour delay in sunrise, the acrophase of cortisol would be delayed. This finding aligns with a study that reported that for each hour delay in a sunrise the cortisol increased by 6.9%.[10] Also, in another study by the same author it was found that for each hour delay in a sunrise there was a 4.8% rise in median cortisol who concluded that the time of sunrise and time of cortisol collection were the most important factors influencing median cortisol.[11] The serum cortisol rose 50% after exposure to bright light from dim light in the early morning, whereas cortisol did not rise in the afternoon for the same bright light. This suggests that the HPA axis response to light is dependent on the time of the day.[12] Also, in our study 20% of subjects had a 09:00 AM cortisol sample value less than 5 μg/dL, which was falsely suggestive of adrenal insufficiency, whereas none of the samples at 06:00 AM had cortisol sample value less than 5 μg/dL.

The cut-off value for adrenal deficiency or sufficiency has been derived from the 08:00 to 09:00 AM cortisol value. When samples are collected during this time period according to the IST in regions of early sunrise, which is after the peak cortisol secretion, they are further away from the cortisol acrophase. This temporal discrepancy can result in lower cortisol levels in samples collected during the usual morning time frames. Thus, it is important to consider the effect of earlier sunrise when interpreting cortisol levels and designing sampling protocols, as the timing of sample collection relative to peak cortisol secretion is an important factor that can influence the observed cortisol levels. The lower basal cortisol levels at 09:00 AM can pose challenges in diagnosis, particularly in ruling out adrenal insufficiency. Misinterpretation of low cortisol levels obtained at 09:00 AM may lead to unnecessary and expensive ACTH stimulation tests to confirm adequate adrenal function. Adapting the sampling time to align with the local sunrise time can provide a more accurate assessment of basal cortisol levels and help in avoiding potential misinterpretation and diagnostic difficulties associated with low measurements. In the present study, we found that IST 06:00 AM is more preferred time for basal cortisol sampling in Guwahati, Assam, rather than between 08:00 AM to 09:00 AM to assess adrenal function.

Additionally, the present study did not show any significant seasonal variation in serum cortisol levels between winter and summer. A previous study reported that cortisol was lower in spring or summer and higher in winter or autumn.[11] The modern lifestyles and artificial lighting adopted in the present day may attenuate the influence of seasonal variations on cortisol secretion.

The present study had less sample size, which impacted the generalisability of the findings. Measuring plasma ACTH simultaneously with serum cortisol could provide a more comprehensive understanding of interpreting the true value of cortisol. This pilot study assessing the effect of sunrise on cortisol levels at different timings has been conducted in a north-eastern part of India. A comparable group from other parts of India will allow for a more comprehensive analysis and comparison of cortisol levels between different regions with the effect of sunrise.

Conclusion

It is essential to consider the influence of earlier sunrise on the circadian rhythm, cortisol secretion and sampling protocols. By recognising the temporal discrepancies and adjusting the sampling time accordingly, healthcare professionals can improve the accuracy of cortisol assessments, avoid

unnecessary testing and ensure appropriate diagnosis and management of adrenal function.

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None.

Authors' contribution

UKS, PN, and AKB conceptualized and designed the study. UKS and AKB supervised the study. PN and AB did data acquisition and statistical analysis. All authors contributed to the study execution, drafted the initial manuscript, and approved the final version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

Data Availability statement

The data supporting the findings of this study can be obtained from the corresponding author upon request.

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