

# Correlation between androstenedione and 17-hydroxyprogesterone levels in the saliva and plasma of patients with congenital adrenal hyperplasia

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## ABSTRACT

**Introduction:** Congenital adrenal hyperplasia (CAH) or adrenogenital syndrome is the most common cause of female ambiguous genitalia. Management of such patients involves medical treatment using glucocorticoids such as hydrocortisone, prednisone or dexamethasone. Monitoring is done by measurement of 17-hydroxyprogesterone (17-OHP) or androstenedione in serum, plasma or saliva. The aim of this study was to develop a system of monitoring steroid treatment in CAH patients using only saliva.

**Methods:** We studied the saliva of 24 CAH patients who received glucocorticoid replacement therapy. The patients were asked to collect saliva upon awakening, and in the afternoon and evening. The levels of 17-OHP and androstenedione in the saliva as well as in serum were then measured by immunoassay.

**Results:** There was a significant positive correlation between 17-OHP in serum and in saliva (R equals 0.929, p-value less than 0.01). A significant positive correlation between androstenedione level in saliva and serum was also found (R equals 0.611, p-value less than 0.01). This study also revealed a significant positive correlation between androstenedione and 17-OHP in serum (R equals 0.647, p-value less than 0.01) and saliva (R equals 0.799, p-value less than 0.01). All patients showed increased level of 17-OHP and androstenedione in the sample collected upon awakening.

**Conclusion:** Determination of salivary androstenedione and 17-OHP in CAH

patients could be a useful alternative to the measurement of these hormones in serum.

**Keywords:** 17-hydroxyprogesterone, ambiguous genitalia, androstenedione, congenital adrenal hyperplasia, saliva

*Singapore Med J 2011; 52(11): 810-813*

## INTRODUCTION

Sexual differentiation follows a specific complex sequence of events. This process starts at around 6–14 weeks of gestation.<sup>(1)</sup> During this period, developmental errors may occur and lead to sexual ambiguity or discordance between chromosomal sex and the appearance of external genitalia. Disorders of sexual development (DSDs) are now classified as 46,XY DSD, 46,XX DSD and chromosomal DSD.<sup>(2)</sup> Congenital adrenal hyperplasia (CAH), or adrenogenital syndrome in the older literature, is the most common cause of ambiguous genitalia in females (46,XX DSD).<sup>(2)</sup> This syndrome occurs due to a mutation in one of the genes encoding enzymes needed for the production of glucocorticoids and/or mineralocorticoids in the adrenal cortex. About 90% of CAH is caused by 21-hydroxylase deficiency, leading to a block in both cortisol and aldosterone production and resulting in an excessive adrenal androgen production causing masculinisation of the genital and urinary structures. A lack of aldosterone and cortisol would lead to adrenal crisis, which could cause severe illness and even lead to death.

A milder, non-life-threatening form of CAH (non-classic form), in which salt loss does not occur, manifests in later childhood or even young adulthood as a result of progressive virilisation.<sup>(3)</sup> Management of CAH patients involves medical treatment in addition to surgery and psychological counselling. Glucocorticoids such as hydrocortisone, prednisone or dexamethasone are the drugs of choice in CAH patients.<sup>(4)</sup> Fludrocortisone, a synthetic mineralocorticoid, is required for salt wasters to retain salt. Follow-up of the treatment is

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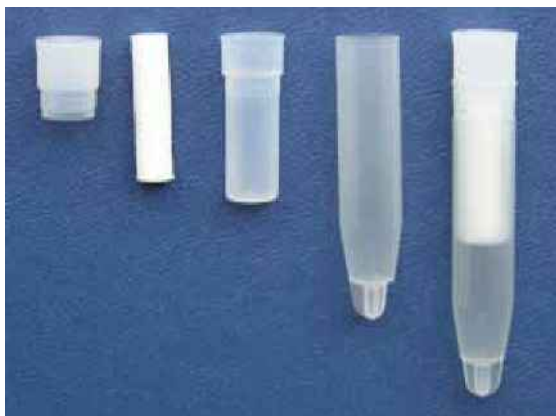
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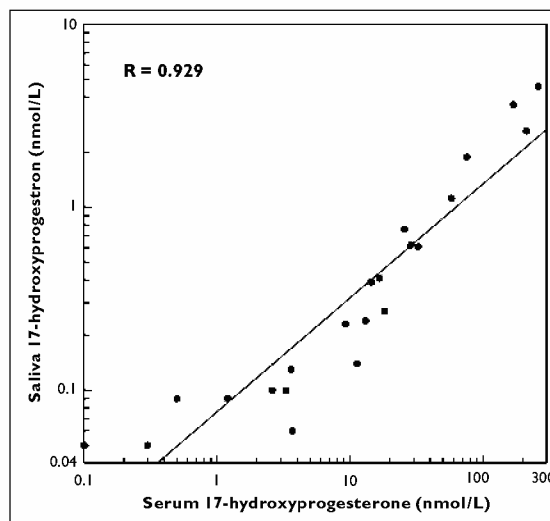
**Fig. 1** Photograph shows the Salivette device for the collection of saliva; from left to right: stopper, cotton swab, cotton swab holder with a hole at the bottom, collection tube and the complete device.

important in order to prevent overtreatment, which causes growth inhibition, and undertreatment, which results in virilisation and increased height velocity.<sup>(5)</sup> The effectiveness of treatment is traditionally monitored by measurement of 17-hydroxyprogesterone (17-OHP), androstenedione and testosterone in serum.<sup>(6)</sup> This means that the invasive procedure of phlebotomy needs to be done every 3–6 months in patients, some of whom are babies or young children.

In this study, we compared steroid hormone levels obtained simultaneously in plasma and saliva in order to develop a system of monitoring steroid treatment in CAH patients using only saliva. This paper aims to make a contribution in the area of CAH treatment by measuring the diurnal rhythm of salivary steroids during treatment with three daily doses of cortisol.

## METHODS

For analysis of steroids in saliva, we collected the saliva of 24 patients with CAH who have relatively high levels of 17-OHP in serum, which would likely result in high levels of salivary 17-OHP. The patients were aged 1.4–16.3 years. All patients received hydrocortisone 12 mg/m<sup>2</sup>/day in three divided doses. Patients with CAH were willing to participate in the study and consented to saliva and blood collection. The diagnoses varied from the severe form of classical CAH to the non-classical form of CAH. All samples of saliva and blood were taken prior to the glucocorticoid treatment. After the procedure was clearly explained to the participants, saliva was collected using a Salivette (Sarstedt®, Nümbrecht, Germany) (Fig. 1) by chewing on the swab. Patients were asked to collect their saliva upon awakening, 30 minutes after waking in the afternoon and in the evening before eating or brushing their teeth, so as to prevent blood contamination. They



**Fig. 2** Graph shows the relationship between saliva and serum levels of 17-OH progesterone collected at the same time.

were also instructed to keep the saliva in the refrigerator and to immediately hand it over during hospital visit at the scheduled time. A duplicate saliva collection was performed during the patients' hospital visit together with peripheral blood collection. The saliva samples collected were then centrifuged and stored frozen at  $-20^{\circ}\text{C}$  until analysis. This study was approved by the local Medical Ethics Committee.

Results are expressed as mean  $\pm$  standard error of the mean (SEM). Differences among hormone levels in samples collected at different times of the day were compared using paired student's *t*-test. Logarithmically transformed concentrations of salivary 17-OHP and androstenedione were correlated with the serum values. Pearson correlation coefficients were calculated using the Statistical Package for the Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA). A *p*-value  $< 0.05$  was considered statistically significant.

## RESULTS

There was a significant positive correlation between 17-OHP in serum and 17-OHP in saliva collected from the same subject at one moment in time ( $R = 0.929$ ,  $p < 0.01$ ) (Fig. 2). As seen in Fig. 3, there was also a significant positive correlation between androstenedione levels in saliva and serum ( $R = 0.611$ ,  $p < 0.01$ ). The androstenedione levels in saliva showed a significant positive correlation with the 17-OH level in saliva ( $R = 0.641$ ,  $p < 0.01$ ) (Fig. 4). This study also revealed a significant positive correlation between androstenedione and 17-OHP in serum ( $R = 0.647$ ;  $p < 0.01$ , data not shown).

A circadian variation of salivary 17-OHP and

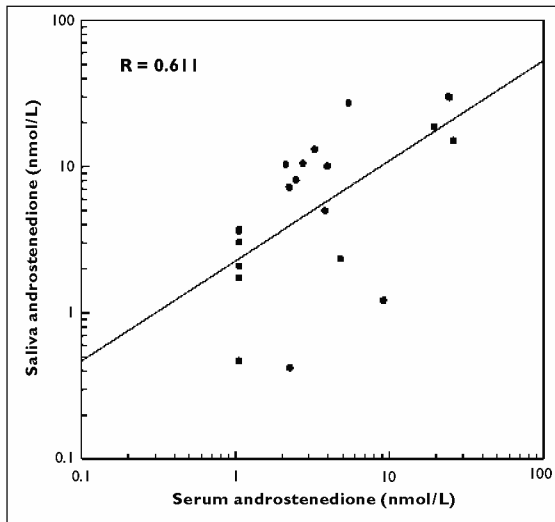


Fig. 3 Graph shows the relationship between the saliva and serum levels of androstenedione collected at the same time.

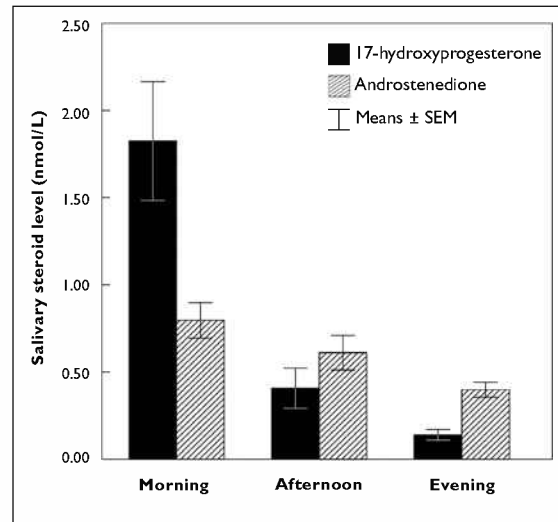


Fig. 5 Graph shows the diurnal rhythm of salivary 17-OH progesterone and androstenedione.

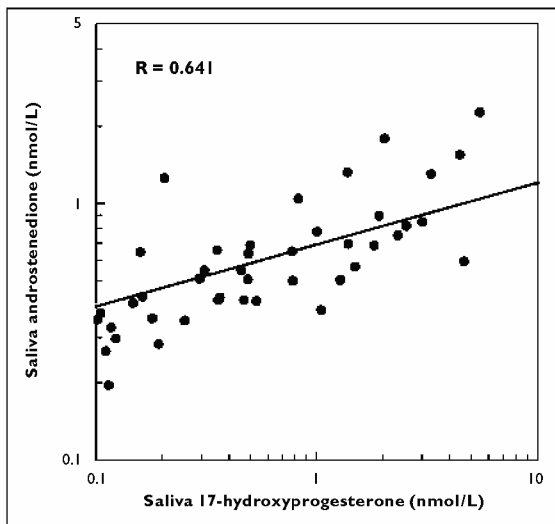


Fig. 4 Graph shows the relationship between the saliva levels of 17-OH progesterone and androstenedione.

androstenedione levels was found in this study (Fig. 5). The levels of 17-OHP were significantly higher in the morning than during the afternoon and evening. Furthermore, the 17-OHP levels in samples collected 30 minutes after awakening ( $3.00 \pm 0.75$  pmol/L) were significantly higher than those at the time of awakening ( $3.55 \pm 0.87$  pmol/L,  $p < 0.05$ ). Concentrations of androstenedione on awakening were greater than those at other times during the day, but significance was reached only when the morning and evening concentration levels were compared.

**DISCUSSION**

Hormone levels are often measured in serum using immunoassays. The result is based on the interpretation of hormone concentrations in a single sample. However, a single serum analysis is not ideal for the study of

hormones that undergo circadian rhythms of secretion. Hormones such as cortisol and 17-OHP can vary in concentrations throughout the day, depending on the time of the day and the physical condition.<sup>(8)</sup> Measurements of salivary 17-OHP in healthy children have revealed that the values tend to be higher in the morning than during the afternoon and evening.<sup>(9)</sup> Within the first minutes after awakening, the salivary cortisol levels rise by 50%–60%. This response was not dependent on the time of awakening, sleep quality or physical activity. After the morning peak, cortisol values decline during the day.<sup>(10,11)</sup> A similar rise of 17-OHP was found in the CAH patients treated with glucocorticoid in this study. Furthermore, all the patients showed relatively high levels of 17-OHP in the samples collected at the time of awakening, probably because saliva was collected before taking the morning dose of glucocorticoid. This observation suggests insufficient suppression of adrenal steroid production during the early morning hours. The values for salivary androstenedione were lower than those for 17-OHP, and gradually fell during the day to its lowest at evening time. There was a circadian rhythm in the concentration of androstenedione in treated patients with CAH, but the level was lower than that for 17-OHP.<sup>(12)</sup>

We observed that 17-OHP was a better marker for treatment evaluation, especially in young patients, as the level of androstenedione in these patients was rather low due to the relatively inefficient adrenal conversion of 17-OHP to androstenedione at a young age. We found a good correlation between the serum and salivary levels of both 17-OHP and androstenedione. These results indicate that the steroid levels in saliva describe the steroid levels in serum. The levels of androstenedione were also correlated with those of 17-OHP. These findings correlate

well with the earlier results of Young et al, who also found that there were high correlations between plasma concentrations of androstenedione and testosterone, as well as with 17-OHP.<sup>(12)</sup>

A strong correlation between the salivary androstenedione profiles and plasma testosterone concentrations was also found in Young et al's study.<sup>(12)</sup> The same correlation between the concentration of androstenedione in saliva and the unbound concentration in plasma was also found in the study of Baxendale et al.<sup>(13)</sup> Similarly, Otten et al found significantly positive correlations between the salivary and plasma values of androstenedione and 17-OHP.<sup>(14)</sup> Measurement of 17-OHP concentrations was performed by Hughes and Read<sup>(15)</sup> in 19 patients with CAH who received hydrocortisone treatment; they found that serial measurement of 17-OHP in saliva provided valuable information in the line of treatment. Arisaka et al<sup>(16)</sup> likewise found good correlation between 17-OHP concentrations in paired saliva and serum samples from the patients and control subjects. Salivary steroid profiles are now widely accepted and used to assess the quality of substitution therapy, providing information on the efficacy of suppressive regimens in the treatment of CAH patients. In addition to 17-OHP, salivary androstenedione is also a direct marker of adrenal androgen secretion. The advantages of the use of salivary steroid profiles include ease of collection of the sample at home and ease of dispatch to the laboratory during regular medical check-up for outpatient monitoring.<sup>(17-19)</sup>

As the levels of steroid hormone in saliva and serum showed good correlation, we can conclude that the determination of salivary androstenedione and 17-OHP levels is a useful alternative to measurements of these hormones in serum for the monitoring of CAH treatment.

#### ACKNOWLEDGEMENT

We thank the members of the Gender Team, Faculty of Medicine, Erasmus Medical Centre and Diponogoro University/Dr Kariadi Hospital for their help in providing the patients for this research.

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