

Salivary versus Serum Testosterone Levels in Boys with Constitutional Delay of Growth and Puberty

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Background

Salivary testosterone (Sal-T) has been established as a noninvasive biomarker in the diagnosis of androgen deficiency in men. However, available data on its utility in adolescent boys with constitutional delay of growth and puberty (CDGP) are not conclusive. Our study was designed to compare salivary versus serum measurements of free testosterone (FT) from the samples collected simultaneously and correlated them with the clinical parameters in boys with CDGP.

Patients and methods

The study enrolled 25 adolescent boys with CDGP and 20 healthy controls matched for age and sex. Weight, height, BMI, testicular volume, bone age, serum follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were assessed. Simultaneous morning saliva and serum samples were obtained for FT measurements by radioimmunoassay and Sal-T by the enzyme-linked immunosorbent assay technique.

Results

Adolescent boys with CDGP had significantly lower Sal-T and serum free-T than normal controls ($P < 0.001$). Sal-T positively correlated with BMI, bone age, testicular volume, serum FSH, LH, and FT. With multiple regression analysis BMI, FSH, LH, and serum FT remained independently correlated with Sal-T. Using the receiver operating characteristic curve, Sal-T was found to be more sensitive than serum FT (76 vs. 69%) in the clinical assessment of boys with CDGP.

Conclusion

Our data shows that Sal-T strongly correlates with FT and provides a sensitive, simple, noninvasive, and diagnostic approach for androgen status in adolescent boys with CDGP.

Keywords:

constitutional delay of growth and puberty, free testosterone, salivary testosterone

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Introduction

Constitutional delay of growth and puberty (CDGP) is a disorder occurring in healthy adolescents who have short stature compared with their peers, with delay in bone growth and puberty [1]. Most boys with CDGP begin to deviate from the normal growth curve before the age of 2 years, and subsequently grow at a relatively normal velocity, and then have a delayed pubertal growth spurt [2]. In boys with CDGP, a testicular volume of 3–4 ml is first reached when they are more than 13.7 years old. The sleep-related luteinizing hormone (LH) increase that characterizes the onset of puberty is normally present in CDGP. The LH response to LH-releasing hormone analogs is intermediate between that of hypogonadal patients and normal pubertal children [3]. CDGP represents the extreme tail of the normal distribution, aggregates in families [4], and is much more common in boys [5].

A suspected diagnosis of CDGP can be confirmed only when puberty and the pubertal growth spurt finally do occur spontaneously. In boys, puberty is characterized

by increasing concentrations of gonadal testosterone driven by increasing concentrations of pituitary gonadotrophins which are, in turn, regulated by the gonadotrophin-releasing hormone released by the hypothalamus [6,7]. A functional defect in any of the components of this hormonal axis directly affects puberty and reproduction.

Circulating testosterone is routinely measured in laboratories for investigation of puberty-related disorders. Normally, testosterone circulates in the plasma either free (2.23%) or bound to serum proteins; sex hormone-binding globulin [8]. Bioavailable testosterone, including both the measurable free and albumin bound fractions, might reflect more accurately the clinical situation when the

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total serum testosterone is borderline or steroid-binding protein levels are impaired [9].

Saliva is a widely accepted sample source for steroid analysis and offers a noninvasive and stress-free alternative to plasma [10]. Salivary testosterone (Sal-T) reflects the free fraction of plasma testosterone that is able to diffuse passively across the salivary glands, independent of the salivary flow rate [11]. Recently Sal-T was suggested as a biomarker for the diagnosis of male androgen deficiency, representing an attractive alternative to plasma [12–14].

Unfortunately, the usefulness of Sal-T was not further investigated specially in boys with CDGP who are frequently referred for endocrine clinics for evaluation. So the aim of this study was to investigate if Sal-T could become a reliable noninvasive tool to screen or assess the androgen status in boys with CDGP.

Patients and methods

The study enrolled 25 adolescent boys with CDGP, from 14 to 16 years of age. Twenty healthy adolescent boys were matched for age in the mid-pubertal and late-pubertal stage (3–5 Tanner stage) as controls. Informed consents were obtained from all participants, and approval was given by the ethics committee of our institution.

The diagnosis of CDGP was based on short stature (height <2 SD below the mean), delayed puberty (onset delayed by >2 SD; has not achieved 4 ml testes until aged 13 years), bone age below the 10th centile for chronological age (delayed by >1.5 years), and absence of other causes of delayed puberty as assessed by routine history taking, physical examination, and biochemical investigations.

BMI was calculated from measurement of the weight in kilograms divided by the square of the height in meters. Pubertal stage was evaluated based on the method of Tanner and Whitehouse [15]. Skeletal age was determined on left wrist radiographs using the Greulich and Pyle method. Bone age delay was noted as the difference between skeletal and chronological age.

Hormonal analysis

Determination of serum free testosterone (FT) was done by direct competitive radioimmunoassay analog tracer [16] and determination of serum LH and follicle-stimulating hormone (FSH) by an automated chemiluminescent immunometric assay [17,18].

Determination of Sal-T: the participants were asked to avoid eating or drinking or brushing their teeth for

30 min before sampling and they were asked to rinse their mouth with cold water for 5 min before sampling. Three milliliters of saliva was collected by direct spitting into glass tubes. The samples were stored at 20°C till the time of the analysis. Sal-T was measured by competitive enzyme-linked immunosorbent assay [19].

Statistical analysis

Data entry and analysis were performed using SPSS statistical package version 17. The results are expressed as the mean±SD. Comparisons of the mean differences between the two groups were performed using Student's *t* test. The *P* value was considered significant if less than 0.05. Pearson's correlation was used to correlate different quantitative variables. Simple regression analysis was performed with Sal-T as the dependent variable and all other parameters as independent variables. Multiple regression analysis was also performed with Sal-T as the dependent variable and BMI, testicular volume, bone age, FSH, LH, and testosterone as independent variables. The receiver operating characteristic (ROC) curve was used to demonstrate graphically the sensitivities and specificities of the different diagnostic tests and cutoff values optimized for sensitivities.

Results

Baseline characteristics of the adolescents with CDGP and age-matched and sex-matched controls are given in Table 1. Adolescent boys with CDGP had significantly lower body weight, height, BMI, testicular volume, and bone age than controls. FSH, LH, FT, and Sal-T levels were significantly lower ($P<0.001$) in adolescent boys with CDGP than controls.

Table 1 Patient characteristics

Characteristics	Adolescents with CDGP (n=25)	Normal control (n=20)	<i>P</i> value
Age (years)	15.1±0.67	15.1±0.72	0.9
Weight (kg)	38.5±3.1	64.3±13.36	<0.001*
Height (cm)	147.8±5.9	169.55±9.9	<0.001*
BMI (kg/m ²)	17.6±0.9	23.4±2.2	<0.001*
Testicular volume (ml)	2.6±0.5	11.6±2	<0.001*
Bone age (years)	12.7±0.5	15.1±0.67	<0.001*
FSH (IU/ml)	0.4±0.08	5.1±0.7	<0.001*
LH (IU/ml)	0.2±0.06	4.3±0.7	<0.001*
Free testosterone (ng/ml)	0.06±0.02	3.5±3.2	<0.001*
Salivary testosterone (pg/l)	0.036±0.012	2.6±2.4	<0.001*

Data are expressed as mean±SD. CDGP, constitutional delay of growth and puberty; FSH, follicle-stimulating hormone; LH, luteinizing hormone. **P* value less than 0.05, significant.

Correlations between Sal-T levels and all other parameters

Sal-T levels were positively correlated with height, weight, testicular volume, bone age, FSH ($P=0.04$), LH ($P=0.038$), and FT ($P=0.039$) (Table 2).

A multiple regression analysis to assess the independent effect of the studied variables (BMI, testicular volume, bone age, FSH, LH, and FT) on Sal-T level was also performed. BMI ($b=0.77$, $P=0.002$), FSH ($b=0.64$, $P=0.03$), LH ($b=0.66$, $P=0.02$), and testosterone ($b=0.41$, $P=0.009$) remained independently correlated with Sal-T levels.

Using the ROC curve, Sal-T was found to be more sensitive than serum FT (76 vs. 52%) in diagnosing

Table 2 Correlation between salivary testosterone and clinical and biochemical parameters in the constitutional delay of growth and puberty group

Parameters	<i>r</i>	<i>P</i> value
Age (years)	0.13	0.5
Weight (kg)	0.62	<0.001*
Height (cm)	0.79	0.002*
BMI (kg/m ²)	0.87	<0.001*
Testicular volume (ml)	0.37	0.049*
Bone age (years)	0.52	0.011*
FSH (IU/ml)	0.44	0.04*
LH (IU/ml)	0.42	0.038*
Free testosterone (ng/ml)	0.43	0.039*

FSH, follicle-stimulating hormone; LH, luteinizing hormone. **P* value less than 0.05, significant.

CDGP based on the clinical criteria, while the specificity of serum FT was 88 versus 69% for Sal-T (Figs 1 and 2).

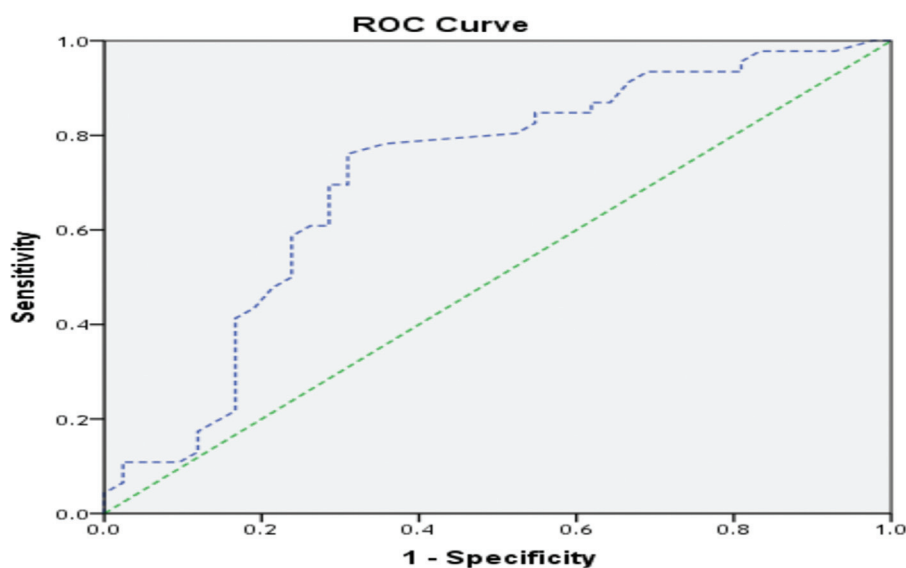
Discussion

Concerns raised by the diagnostic use of saliva are many and variably appreciated. The present study was made to investigate the reliability of a noninvasive procedure for the measurement of testosterone in saliva as a diagnosing tool in cases of CDGP, and comparing its utility with serum FT.

Our results showed a significantly lower Sal-T levels in adolescent boys with CDGP in comparison with the control group ($P<0.001$). Moreover, Sal-T levels were positively correlated with FT ($P=0.039$). These results are in good agreement with those of other studies in normal boys conducted by Umehara *et al.* [20] indicate that Sal-T correlates well with FT in boys. Furthermore, this adds to the previous literature confirming that Sal-T correlates with FT as described in healthy men [21–23].

While Sal-T is considered a mirror of FT in normal adolescent boys or those with low T as in boys with CDGP and men with hypogonadism, it is of greater diagnostic use in hyperandrogenic conditions as hirsutism, where it was a reliable method than any of the currently used serum androgen assays [24,25].

Figure 1

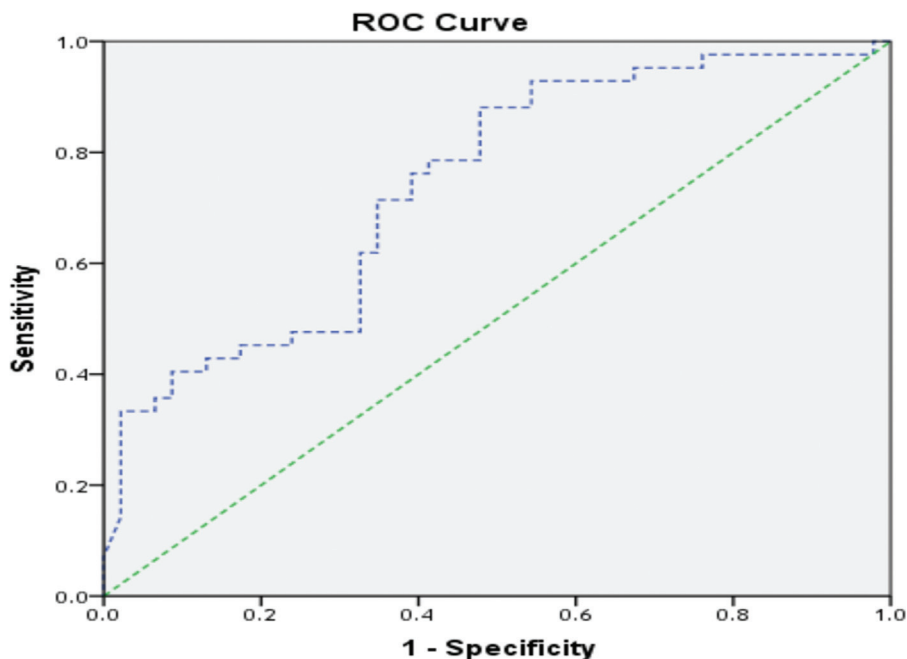


Diagonal segments are produced by ties.

Salivary testosterone

Salivary testosterone. ROC, receiver operating characteristic.

Figure 2



Diagonal segments are produced by ties.

Free testosterone

Free testosterone. ROC, receiver operating characteristic.

This study showed a statistically significant correlation between Sal-T and LH and FSH levels ($r=0.42$, $P=0.038$ and $r=0.44$, $P=0.04$), which is considered the main biochemical standard in the assessment of androgen status. Also a significant correlation was found between Sal-T and BMI, testicular volume, and bone age. These results are in agreement with the findings seen in normal men and those with hypogonadism [21,26–28] and indicate the relationship between Sal-T and the clinical parameters of puberty as seen with serum testosterone [29]. Despite the limitations of using saliva, like being affected by changes in salivary PH and flow, its use is increasing so much [30]. This goes with Chiappin *et al.* [31], who stated that it is likely that the use of saliva in assays will continue to expand, thus providing a new tool of investigation for physiologic as well as pathophysiologic conditions.

Finally, in our study, the reliability of Sal-T was documented using the ROC which showed that Sal-T was found to be more sensitive than serum FT (76 vs. 69%) in diagnosing boys with CDGP, while the specificity of serum FT was 88 versus 52% for Sal-T. The constellation of clinical parameters and the documentation of the sensitivity of measurement of Sal-T might indicate that determination of Sal-T is a reliable method to detect changes in the concentration of the biologically active testosterone in the serum.

The importance of this study is that Sal-T measurement if really diagnostic would be a noninvasive approach to screen androgen status in boys with CDGP and abolish the use of other markers using the venous sample.

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Conflicts of interest

There are no conflicts of interest.

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