

# Salivary Versus Serum Approaches in Assessment of Biochemical Hyperandrogenemia

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## ABSTRACT

**Background:** Biochemically, hyperandrogenism is established by elevated circulating levels of free or total serum testosterone and an increased free androgen index. Approximately, 60-80% of women with polycystic ovarian syndrome (PCOS) demonstrate elevated circulating androgen levels. **Aim:** This prospective observational randomized study was to investigate the likelihood of using the salivary luteinizing hormone (LH), free testosterone (FT) and dehydroepiandrosterone sulfate (DHEAS) levels instead of serum values in diagnosing biochemical hyperandrogenemia in women with PCOS. **Subjects and Methods:** This study was conducted on 75 women having PCOS in addition to 20 normal fertile women (control group). Venous blood and salivary samples were taken in the 3<sup>rd</sup> day of the cycle to measure LH and FT and DHEAS levels. **Results:** Biochemical hyperandrogenemia prevails in 40% of women with PCOS. Salivary levels of LH, FT and DHEAS correlate with their corresponding serum values, with a higher sensitivity of salivary more than serum approach. **Conclusion:** Saliva provides a sensitive, simple, reliable, non-invasive and uncomplicated diagnostic approach for biochemical hyperandrogenemia.

**KEY WORDS:** Biochemical hyperandrogenemia, dehydroepiandrosterone sulfate, luteinizing hormone, polycystic ovarian syndrome, saliva, testosterone

## INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a widely spread disease among females during their reproductive period. One of the criteria on which the diagnosis of PCOS is built on, is the assessment of testosterone hormone (T), which is often elevated in such cases.<sup>[1]</sup> It is estimated that 60-80% of women with PCOS demonstrate elevated circulating androgen levels.<sup>[2]</sup>

Biochemically, hyperandrogenism is established by elevated circulating levels of free or total serum testosterone, androstenedione and an increased free androgen index.<sup>[3]</sup>

Saliva, which offers a non-invasive and stress-free alternative to plasma and serum, is a widely accepted sample source for analysis of steroids and of certain amines and peptides. In recent years, numerous publications have described the

use of salivary hormone analysis in many fields of clinical and basic research.<sup>[4-6]</sup> Although saliva has not yet become a mainstream sample source for hormone analysis, it has proven to be reliable and in some cases, even superior to other body fluids. Nevertheless, much effort will be required in this approach to receive acceptance over the long-term, especially by clinicians.<sup>[7]</sup>

Sex steroids have been analyzed successfully in saliva for years. In assessing the ovarian cycle, saliva samples have been demonstrated to enable differentiation between the follicular and luteal phase for both estradiol and progesterone.<sup>[8]</sup>

The previous studies showed that salivary T gives a useful indication of levels of biologically available androgen in hyperandrogenic women.<sup>[9]</sup> Testosterone is secreted in saliva in the free form without.<sup>[10]</sup>

The aim of this work was to determine the likelihood of using saliva more than serum in diagnosing biochemical hyperandrogenemia in women with PCOS.

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## SUBJECTS AND METHODS

This prospective observational cohort study was conducted on 75 women having PCOS, selected from those attending the Department of Obstetrics and Gynecology, Tanta University Hospital, during the period from November 2011 to April 2012. All PCOS cases fulfilled the Rotterdam revised diagnostic criteria of polycystic ovary syndrome<sup>[1]</sup> in addition to 20 normal fertile women (control group).

All patients included within the study were counseled thoroughly about the procedure, including its value and hazards, and aim for this study. After this, a written consent was obtained and signed by the patient in accordance the declaration of Helsinki. Moreover, the ethical committees at the department approved the protocol.

The most important inclusion criteria were: Age between 20 and 35 years, complaining of primary infertility, having no ovulation induction drugs during at least the last 3 months before the procedure, body mass index between 25 and 29.9, absence of other causes of infertility evidenced by semen analysis and hysterosalpingography.

The most important exclusion criteria were: Age  $\geq$  35 years, history of medical or endocrinological disorders, history of abdominal or pelvic surgical operations, history of ovarian diseases; tumors, pathological cysts, endometriosis, or tuboovarian abscess, history of dental and oral diseases or operations, patients receiving any medications, especially hormonal therapy.

### Methods

The following methods were undertaken for each patient Full history taking and thorough general and local examination. Venous blood sample was taken in the 3<sup>rd</sup> day of the cycle, either spontaneous or progestin-induced withdrawal bleeding, to measure luteinizing hormone (LH), free testosterone (FT) and dehydroepiandrosterone sulfate (DHEAS) levels. Salivary sample was collected to measure LH, FT and DHEAS levels in the 3<sup>rd</sup> day of cycle by the enzyme-linked immunosorbent assay.

Analysis of the results was performed using the mean, standard deviation, *t*-test, analysis of variance, linear correlation according to the methods described by Petrie and Sabin using statistical product and service solutions] software statistical computer package version 12.<sup>[11]</sup> This software allows statistical analysis, data management and data documentation.  $P < 0.05$  was considered as significant.

## RESULTS

The percentage of the biochemical hyperandrogenemia among the studied cases with PCOS was 40%.

Both serum and salivary LH were significantly higher in PCOS patients than that of the control cases ( $P$  values were  $<0.001$ , in favor of salivary results), as depicted in Tables 1 and 2.

FT in both the serum and saliva was significantly higher among the PCOS women than the corresponding values from the control  $P = 0.01$  and  $<0.01$  respectively, in favor of salivary findings.

DHEAS in both the serum and saliva was insignificantly higher among the PCOS women than the corresponding values of the control.

Table 3 and Figures 1-3 depict the presence of a statistically positive correlation between the salivary and serum LH and FT level ( $P = 0.02$ ). Similarly, there is a statistically positive correlation between the salivary and serum LH ( $P = 0.001$ ), the salivary and serum level of FT ( $P = 0.001$ ) and between the salivary and serum DHEAS ( $P = 0.001$ ).

**Table 1: Serum levels of LH, testosterone and DHEAS in control and PCOS subjects**

	Serum LH (mIU/ml)		Serum FT (ng/dl)		Serum DHEAS (ug/ml)	
	Control	PCOS	Control	PCOS	Control	PCOS
No. of cases	20	75	20	75	20	75
Mean	3.62	6.36	35.82	48.79	260.8	272.3
SD	2.84	3.43	20.41	24.12	98.8	100.5
t	3.661		2.426		0.461	
P	0.00036		0.01087		0.32504	

LH – Luteinizing hormone; DHEAS – Dehydroepiandrosterone sulfate; PCOS – Polycystic ovarian syndrome; SD – Standard deviation; FT – Free testosterone

**Table 2: Salivary levels of LH, testosterone and DHEAS in control and PCOS subjects**

	Salivary LH (mIU/ml)		Salivary FT (ng/dl)		Salivary DHEAS (ug/ml)	
	Control	PCOS	Control	PCOS	Control	PCOS
No. of cases	20	75	20	75	20	75
Mean	2.82	3.63	67.90	72.70	0.63	2.02
SD	0.34	0.54	6.82	9.21	0.91	1.70
t	8.238		2.582		4.916	
P	0		0.00657		0	

LH – Luteinizing hormone; DHEAS – Dehydroepiandrosterone sulfate; PCOS – Polycystic ovarian syndrome; SD – Standard deviation; FT – Free testosterone

**Table 3: Correlation between serum and salivary LH, FT and DHEAS**

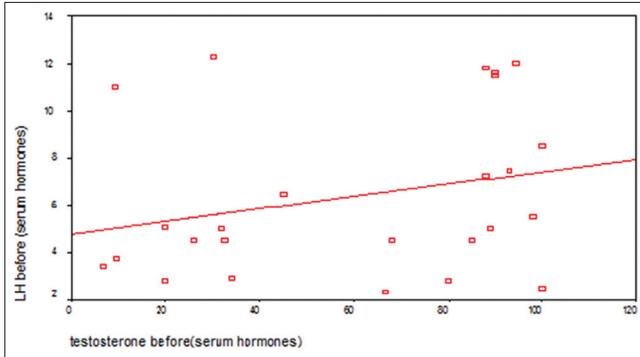
	r	P value
Serum LH	0.874	0.001
Salivary LH		
Serum FT	0.562	0.001
Salivary FT		
Serum DHEAS	0.542	0.001
Salivary DHEAS		

Notice that there is a statistically positive correlation between the salivary and serum LH, FT and DHEAS. LH – Luteinizing hormone; DHEAS – Dehydroepiandrosterone sulfate; FT – Free testosterone

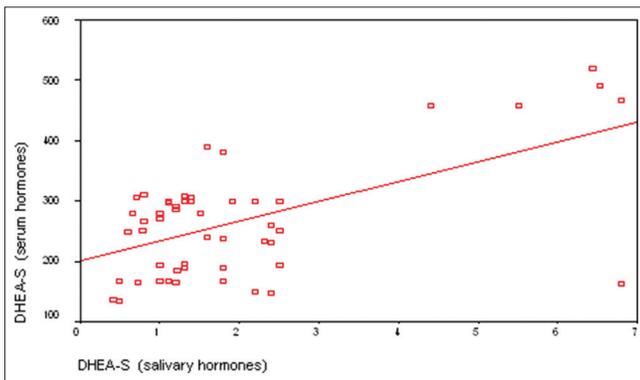
Figure 3 reveals that the receiver operating character proves that the accuracy of measuring salivary testosterone and LH is higher than the corresponding serum values.

## DISCUSSIONS

Polycystic ovary syndrome is one of the most important causes of female hyperandrogenemia, reflected by elevated circulating androgen levels, particularly testosterone. Both ovaries and the adrenal glands secrete androgens.<sup>[12]</sup>



**Figure 1:** Correlation between the salivary and serum LH, testosterone (LH – Luteinizing hormone)



**Figure 2:** Correlation between the salivary and serum DHEAS (DHEAS – Deydroepindrosterone sulphate)

The typical endocrine abnormalities in anovulatory women with PCOS are raised serum concentrations of androgens and LH, with normal or slightly suppressed serum follicle stimulating hormone levels. Approximately, 50% of cases with PCOS have raised LH concentrations and approximately 30% with PCOS have. An elevated total testosterone on spot testing.<sup>[13]</sup>

The results from this standing research revealed that biochemical hyperandrogenemia was present in 40% of our patients with PCOS, which in turn supports the previous suggestion.<sup>[2]</sup> This finding varies from that of Huanget and associates who reported that the overall prevalence of hyperandrogenemia in PCOS was 75.3%.<sup>[14]</sup>

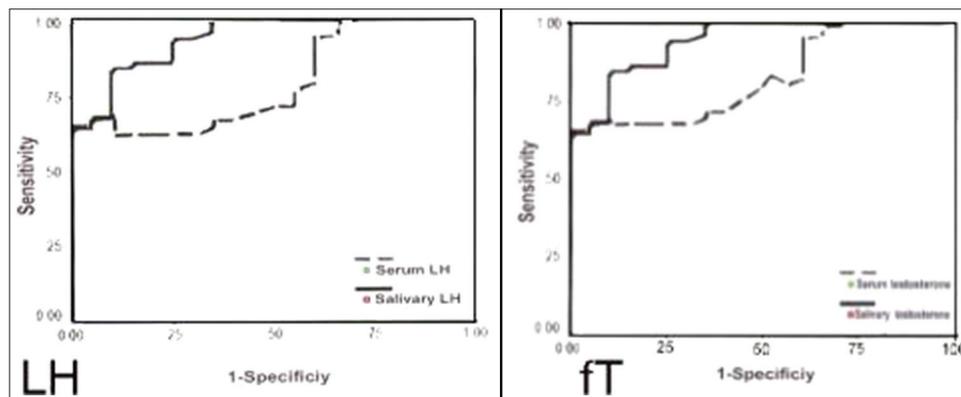
Salivary androstenedione/salivary testosterone ratio may be a good indicator of hyperandrogenism in women. We also confirm that measurement of androstenedione in plasma may be useful in making a diagnosis of PCOS.<sup>[15]</sup>

Omnia-Youssef concluded that determination of salivary testosterone is a reliable method to detect changes in the concentration of available biologically active testosterone in the serum. Salivary testosterone provides a sensitive, simple, reliable, non-invasive and uncomplicated diagnostic approach for PCOS.<sup>[16]</sup>

The contemporaneous study proved the presence of a significant positive correlation between the salivary and serum testosterone ( $r = 0.506$  and  $P = 0.001$ ). These results match with those of many other authors.<sup>[8]</sup>

In addition, we noticed that serum and salivary testosterone were also found to correlate with LH in cases of PCOS. This finding agrees with that of other authors.<sup>[17]</sup>

Finally, we conclude that saliva provides a sensitive, simple, reliable, non-invasive and uncomplicated diagnostic approach for biochemical hyperandrogenemia.



**Figure 3:** The receiver operating character of salivary versus serum LH and FT. (FT – Free testosterone LH – Luteinizing hormone)

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