

VALIDITY OF SALIVARY TESTOSTERONE MEASUREMENTS IN HIRSUTE WOMEN BEFORE AND AFTER SPIRONOLACTONE THERAPY

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Material and Methods

The material of study comprised 21 female patients complaining of hirsutism. Their ages ranged from 19 to 35 years (mean 22.6 ± 2.52) and the weight ranged from 53 to 95 kgm (mean 68.2 ± 10.47). Ten carefully selected normal matched females were chosen to act as a control group. Both patients and controls were subjected to detailed history, thorough clinical examination, and radiological examinations including pelvi-abdominal ultrasonography and plain X-ray skull.

Laboratory investigations were carried out in the early follicular phase of the menstrual cycle. Blood sample was taken in the morning and serum separated. A sample of saliva was collected at the same time after thorough rinsing of the mouth, and centrifuged at 1500 Xg for 15 minutes. The clear

supernatant and the serum sample were preserved at -20°C until time of analysis. Spironolactone was administered by all patients in a dose of 100 mg/day from 4th to 22nd day of the menstrual cycle. Serum and salivary samples were again obtained. Measurement of serum free and salivary testosterone was done by RIA using the COAT-A count testosterone kit supplied by Diagnostic Products Corporation; 5700 West 96th street, Los Angeles.

Hirsutism was graded according to the score of Abraham et al. (1976), while clinical improvement was assessed according to Pittaway et al. (1985).

Results

Are shown in tables 1, 2, 3, 4, 5 and 6.

Discussion

In the present study FT and Sart levels were insignificantly higher in patients with other manifestations of hyperandrogenism (Table 3) than those with hirsutism only ($P>0.05$). Also they were significantly lower ($P<0.05$) in patients with duration less than 3 years than the corresponding values

Serum FT and SAT levels in the present study correlated with body mass index (BMI) (Table 2). Similar results were reported for serum FT by Mathur (1983) and for SAT by Ruitabar-men et al. (1987). This could be attributed to reduction of SHBG levels in obese women (Dunkel et al., 1985).

SAT was increased in 19 hirsute patients (90.48%), while 2 patients (9.52%) had normal SAT values. SAT levels in all patients far exceeded those of serum FT at the individual level of each patient. These data are in agreement with previous authors who examined with plasma-bound T is biologically available to tissues and that salivary glands can metabolize androstenedione (A) to T, a process which is exaggerated in hirsute women (Rey et al., 1986).

Previous data showing that the severity of hirsutism is related to the degree of hyperandrogenemia (Ruttiainen et al., 1987).

In this study, there was a significant increase ($P<0.001$) in serum free T and salivary T in patients as compared to controls (Table 1). These results are in agreement with previous studies (Givens, 1985; Schwartz & Fink, 1985; Board & Schiff, 1986 and Rittmaster et al., 1987), and support the concept that excess androgens is important in the pathogenesis of hirsutism and that SAT is elevated in hirsutism and those having mild hirsutism ($P<0.001$) serum FT and SAT when compared with those having mild hirsutism ($P<0.001$). This is in accordance with the group. This is in accordance with the control group. This is in accordance with the group.

Measurement of salivary T, proved to be a useful index of levels of biological availability and hyperandrogenic women and hyperandrogenic women (Buxbaum et al., 1983).

Hirsutism is the growth of terminal hair occurring in the face or the body of a woman in a pattern more typical of that seen in men (Biffignandi et al., 1984). It results from a subtle excess of androgens (Hatch et al., 1981), as hyperandrogenemia is present in up to 90% of hirsute females (Braitwaite

in patients with duration more than 3 years (Table 4). On the other hand, all the patients with short duration had mild hirsutism. The relation of androgen level to duration and severity of hirsutism had been recorded by Toscano et al. (1983).

Out of the 21 patients under study, fourteen (66.7%) had cystic ovarian changes (Table 5). These patients had insignificantly higher ($P>0.05$) serum FT and SaT levels than patients without cystic changes and had significantly higher serum FT ($P<0.001$) and SaT ($P<0.05$) as compared to control, a finding previously observed by many authors (Cumming et al., 1982; Evans & Burke, 1986 and Ruutiainen et al., 1987).

In response to spironolactone therapy 7 of 14 patients who completed the trial fulfilled the criteria for satisfactory clinical response (Pittaway et al., 1985). The mean level of both serum FT and SaT were significantly reduced ($P<0.05$) to means of -25.9% and -32.3% of basal levels respectively after SL therapy (Table 6). Similar results were reported for serum FT by Goodfellow et al. (1984); Muhlemann et al. (1986) and Ylostalo et al. (1987) and for SaT by Baxendale et al. (1982).

In the present study, clinical improvement was strongly correlated with lowering of both serum FT and SaT levels, a finding previously reported by many authors (Lobo et al., 1985 and Ylostalo et al., 1987). Patients with higher initial serum FT and SaT levels had more manifest reductions of these hormonal parameters. This is in agreement with other studies (Shapiro & Evron, 1980; Baxendale et al., 1982 and Cumming et al., 1982). Also, patients with mild hirsutism were clinically less responsive (66.11%) than those with either moderate (80%) or severe hirsutism (76.67%). This result is consistent with the result obtained by Tremblay (1986) that patients with mild hirsutism are less responsive, which may be explained in part by the initially lower T level with consequent less T reduction.

In the present study a significantly positive correlation was observed between SaT and serum FT in all hirsute patients ($r = + 0.85$, $P<0.001$), in control group ($r = + 0.77$, $P<0.01$) and in the 14 patients who completed the trial, both before ($r = + 0.84$, $P<0.001$) and after treatment ($r = + 0.82$, $P<0.001$). This finding was previously reported by many authors (Smith et al., 1979; Khan et al., 1984 and Rey et

For these reasons, SAT measure-
ments could be safely used for as-
sessing the androgenic status and
monitoring of drug therapy.

In addition to its sensitivity in assessing the androgenic status in hirsute females and correlating with the changes in serum FT levels in all the circumstances studied, salivary tests that sampling is a simple, non-invasive, stress free process, avoiding undue disturbance of the endocrine system and the patient can collect the sample by herself.

Conclusion

controls. Serum free testosterone (FT_T) and salivary total testosterone (Sat) were measured by radioimmunoassay (RIA) before and after a course of spinal manipulation therapy starting from the 4th to the 22nd days of the menstrual cycle. Salivary testosterone parallelled changes in serum free testosterone with a significant positive correlation in all circumstances studied, suggesting that SAT measurements could be used as a safe alternative to serum sampling with the added advantages of being simple, non-invasive and avoiding undue disturbances of endocrinological system.

This study was conducted on ten normal-ty-one hirsute females and ten normal-

SUMMARY

Salivary testosterone seems to relate to the bioavailable fraction of the hormone. It is less variable and less subject to episodic and cyclical variations than serum FT, so it facilitates more dynamic tests of hormone function and assessment of biological rhythms and disturbances of endocrine system (Riad et al., 1983). In addition to the ease of sample collection, it provides one of the best hormonal indices for the severity of hirsutism and correlates strongly with the grade of facial hirsutism and hair growth in the hor monal areas and on the whole body. Moreover, SAT concentrations far exceed those of serum FT due to T concentrations in salivary glands (Rey et al., 1986; Ruatainen et al., 1987). So, the human technical errors (Rey et al., 1986; Ruatainen et al., 1987) that are inherent in serum FT estimation are reduced.

the applicability of SAT measurement as a possible alternative to serum FT and Ruuttilainen et al., 1987). In 1986, and gives strong support to the applicability of SAT measurement as a possible alternative to serum FT and Ruuttilainen et al., 1984 or gonadal function (Khan et al., 1984 and Ruuttilainen et al., 1987).

Table (1) : Comparison of patients with different grades of hirsutism to the control group.

Group	Data	Serum FT (pmol/L)	Salivary T (nmol/L)
Control group (10 cases)	Mean ± S. D.	12.62 4.04	0.46 0.12
Mild hirsutism (15 cases)	Mean ± S. D. t	31.39 9.95 6.54**	0.71 0.24 3.44*
Moderate to severe hirsutism (6 cases)	Mean ± S. D. t t'	57.67 8.84 11.77** 5.93*	1.18 0.44 7.18** 5.63**
All hirsute patients (21 cases)	Mean ± S. D. t	38.90 15.30 7.35**	1.01 0.58 4.16**

t = Compared to control.

t' = Compared to patients with mild hirsutism.

* = Significant ($P < 0.05$).

** = Highly significant ($P < 0.001$).

Table (2) : Effect of body mass index (BMI) on hormone levels.

Group	Data	Serum free T (pmol/L)	Salivary T (nmol/L)
Control group (10 cases)	Mean ± S. D.	12.62 4.04	0.46 0.12
BMI less than 25 kgm/m ² (9 cases)	Mean ± S. D. t	28.97 11.00 4.21**	0.59 0.15 2.07
BMI more than 25 kgm/m ² (12 cases)	Mean ± S. D. t t'	46.41 13.79 8.08** 3.22	1.33 0.58 5.07** 4.23**

t = Compared to control.

t' = Compared to patients with BMI < kgm/m².

* = Significant t (at $P < 0.05$).

** = Highly significant t (at $P < 0.001$).

** = Highly significant (at $P < 0.001$).* = Significant (at $P < 0.05$).† = Compared to patients with duration < 3 years.

‡ = Compared to control.

Group	Data	Serum free T ($\mu\text{mol/L}$)	Salivary T ($\mu\text{mol/L}$)
Control group	Mean	12.62	0.46
Hirsutism without manifestations	F.S. D.	4.04	0.12
Hirsutism with manifestations	Mean	35.09	0.98
Hirsutism with other manifestations	F.S. D.	13.12	0.41
Hirsutism with drogenism	Mean	42.36	1.05
Control group	Mean	12.62	0.46
DURATION LESS THAN 3 YEARS	Mean	26.07	0.65
DURATION MORE THAN 3 YEARS	Mean	50.55	1.35
DURATION MORE THAN 3 YEARS (11 cases)	†	11.19*	4.89**
DURATION MORE THAN 3 YEARS (11 cases)	F.S. D.	10.41	0.59
DURATION MORE THAN 3 YEARS (11 cases)	Mean	50.55	1.35
DURATION MORE THAN 3 YEARS (11 cases)	†	6.18*	3.62*

Table (4) : Effect of duration of hirsutism on hormone levels.

** = Highly significant (at $P < 0.001$).* = Significant (at $P < 0.05$).

† = Compared to hirsute patients without other manifestations.

‡ = Compared to control.

Group	Data	Serum free T ($\mu\text{mol/L}$)	Salivary T ($\mu\text{mol/L}$)
Control group	Mean	12.62	0.46
Hirsutism without manifestations	F.S. D.	4.04	0.12
Hirsutism with hypoadrogenic manifestations	Mean	35.09	0.98
Hirsutism with other manifestations	F.S. D.	13.12	0.41
Hirsutism with drogenism	Mean	42.36	1.05
Hirsutism with hypoadrogenic manifestations (10 cases)	†	5.18**	3.85**
Hirsutism with other manifestations (10 cases)	F.S. D.	16.29	0.70
Hirsutism with drogenism (11 cases)	Mean	5.86**	2.75*
Hirsutism with other manifestations (11 cases)	†	1.13	0.28

hypoadrogenism versus control group.

Table (3) : Comparison of patients with and without other manifestations of

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Table (5) : Comparison of hirsute patients with evident cystic ovarian changes and those with idiopathic hirsutism (IH) to the control group.

Group	Data	Serum free T (pmol/L)	Salivary T (nmol/L)
Control group (10 cases)	Mean ± S. D.	12.62 4.04	0.46 0.12
Patients with IH (7 cases)	Mean ± S. D. t	32.49 9.70 5.12**	0.86 0.26 3.80*
Hirsute patients with cystic ovarian changes (14 cases)	Mean ± S. D. t t	42.10 16.53 6.41** 1.67	1.09 0.67 3.44* 1.13

t = Compared to control.

t' = Compared to patients with IH.

* = Significant t (at P < 0.05).

** = Highly significant t (at P < 0.001).

Table (6) : Comparison of hormone levels in hirsute patients before and after spironolactone treatment to the control group.

Group	Data	Serum free T (pmol/L)	Salivary T (nmol/L)
Control group (10 cases)	Mean ± S. D.	12.62 4.04	0.46 0.12
Hirsute patients before treatment (14 cases)	Mean ± S. D. t	38.68 15.60 5.98**	1.09 0.66 3.49*
Hirsute patients after treatment (14 cases)	Mean ± S. D. t	27.76 12.11 4.33**	0.67 0.36 2.03

* = Significant t (at P < 0.05).

** = Highly significant t (at P < 0.001).

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