Salivary androgens' measurement as a useful tool in diagnosis and determination of the effectiveness of metformin treatment in women with polycystic ovary syndrome

Ocena stężenia androgenów w ślinie jako przydatne narzędzie diagnostyczne u kobiet z zespołem policystycznych jajników leczonych metforminą

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The measurement of testosterone concentration is important in the diagnosis and management of PCOS. The most of plasma testosterone assays show poor sensitivity and accuracy in female ranges. The alternative method of plasma measurement is salivary assay. We investigated whether salivary androgens – androstendione and testosterone (salA, salT) are useful as a diagnostic marker for diagnosis and monitoring of treatment. Twenty six women with PCOS underwent assessments at baseline, and after 3 months of the therapy. High correlation between salivary androstendione and free serum androstendione estimated by EQ (r=0.85, p<0.01) (A EQ) and total androstendione in serum (r=0.84, p<0.01) has been shown in patient with PCOS before metformin treatment. After 3 months' therapy the correlation was still significant (respectively r=0.77, p<0.05; r=0.66, p<0.05). Correlation between salA/salT and A EQ, T EQ was respectively r=0.73, p<0.01; r=0.62, p<0.01 in patients before metformin therapy and respectively r=0.55, p<0.01 and r=0,31, p<0.01 in patients after metformin therapy. Salivary androgens may be indicators of hyperandrogenism in women and diagnostic markers in monitoring of treatment in women with PCOS.

Słowa kluczowe:
zespół policystycznych jajników, testosteron w ślinie, androstendion w ślinie, hiperandrogenizm, zaburzenia owulacji

Ocena stężenia testosteronu jest ważnym elementem w diagnostyce i monitorowaniu leczenia kobiet z zespołem policystycznych jajników (PCOS). Większość dostępnych testów diagnostycznych do oceny stężenia testosteronu w surowicy charakteryzują sie niską czułością szczególnie u kobiet. Alternatywą do oceny stężenia testosteronu w surowicy może być analiza w ślinie. Celem pracy była ocena przydatności badania stężenia androgenów w ślinie w diagnostyce i monitorowaniu leczenia. W badaniu wzięło udział 36 kobiet z PCOS- ocena przed leczeniem oraz po 3 miesiącach terapii.Wykazano wysoką korelację pomiędzy stężeniem androstendionu w ślinie a wolnym androstendionem w surowicy wyznaczonym za pomocą metody dializy równowagowej (r=0.85, p<0.01) (A EQ) i androstendionem całkowitym w surowicy (r=0.84, p<0.01) u kobiet z PCOS przed rozpoczęciem leczenia. Po 3 miesiącach terapii korelacja była nadal wysoka (odpowiednio r=0.77, p<0.05; r=0.66, p<0.05). Korelacja pomiędzy salA/salT i A EQ, T EQ wynosiła r=0.73, p<0.01; r=0.62, p<0.01 u kobiet przed rozpoczęciem terapii i odpowiednio r=0.55, p<0.01 i r=0,31, p<0.01 u pacjentek leczonych metforminą. Stężenie androgenów w ślinie może być wskaźnikiem hiperandrogenizacji u kobiet z PCOS na etapie postawienia rozpoznania jak i w ocenie skuteczności leczenia.

Key words:
polycystic ovary syndrome, salivary testosterone; salivary androstendione, hyperandrogenism, anovulation

Introduction

Polycystic ovary syndrome (PCOS) is known as one of the most common endocrinopathies affecting 4% to 7% of women in reproductive age [1,2]. PCOS is characterized by oligo-and/or amenorrhea, fertility problems and/or hyperandrogenism [1,2,3]. According to ESHRE/ASMR consensus PCOS can be diagnosed if at least two out of three following criteria are fulfilled: oligo/anovulation, clinical or biochemical hyperandrogenism, and/or polycystic ovaries found by ultrasound imaging, after exclusion of other causes of menstrual irregularities and hyperandrogenemia [4]. Hyperandrogenism is clinically manifested by hirsutism, persistent acne, alopecia and biochemical abnormalities in blood tests, such as increased level of total (T) and free testosterone (fT) [5,6,7]. Commonly, hyperandrogenemia in PCOS women can be assessed by measuring the level of T and/or fT [8,9]. Androstenedione (A), which is synthesized in the adrenal cortex and ovarian theca cells, may be also used for diagnosing hyperandrogenemia [10]. The pathogenesis of PCOS remains unclear. The syndrome is characterized by an increased frequency of luteinizing hormone (LH) pulse favoring the androgen production by ovarian theca cells and increased 17-alfa-oestradiol conversion in granulose cells. Insulin also plays a role in the pathogenesis of PCOS, acting synergically with LH on theca cells, reducing the sex hormone binding globulin and thus increasing the biologically active androgen levels. In addition, various paracrine and autocrine factors mediate the effect of LH and insulin [11]. Hyperinsulinemia may promote abnormal ovarian androgen secretion and abnormal follicular development leading to dysfunctional ovarian and menstrual activity [11]. Management of women

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with PCOS depends on the symptoms. These could be menstrual disorders, or androgen-related symptoms. Weight loss alone can improve the endocrine profile, normalize menstrual cycles and increase the likelihood of ovulation and pregnancy. The treatment of obesity includes modifications in lifestyle (diet and exercise) and medical and surgical treatment.

The biguanide, metformin, the most widely prescribed insulin sensitizer in the management of type 2 diabetes, can improve insulin resistance and imbalance of endocrine hormones [12]. It may have an additional benefit of improving some features of metabolic syndrome such as dyslipidemia, hypertension and obesity [13]. Administration of metformin has also beneficial role in lowering serum testosterone level by exerting its action over serum insulin and increasing insulin sensitivity of tissues in PCOS [14,15].

The measurement of testosterone concentration is important in the diagnosis and management of PCOS [16]. However, there are several limitations to its use. Most of immunoassay-based testosterone level measurements (ELISA, RIA) are not designed or validated for relatively low plasma levels normally presented in women [9]. The most of testosterone assays show poor sensitivity and accuracy in female ranges [17]. Moreover, these ranges of testosterone concentration in women usually do not consider the age and are wide.

Circulating plasma levels of testosterone in women also vary according to reproductive maturity, phase of the menstrual cycle or time of day [18]. Testosterone levels in hyperandrogenic women, even those with severe signs of hyperandrogenemia, such as hirsutism or acne, are usually within reference ranges [19]. In about 20–40% of patients with PCOS laboratory results fail to identify biochemical hyperandrogenism, so the results are underestimated [18,20]. Methods improving the performance of testosterone immunoassays, such as extraction of testosterone and chromatographic purification before the assay, are not used in commercial setting. The alternative method of plasma testosterone measurement is salivary testosterone assay. Salivary testosterone represents the unbound or bioavailable plasma fraction of testosterone, therefore concentrations are much lower than those in serum [21]. The assessment of salivary androgens has practical advantages: sampling is non-invasive, and the test is easy to use.

The aim of the study was to determine the usefulness of salivary androgens as new diagnostic marker for diagnosis and monitoring of treatment of PCOS patients receiving metformin.

The protocol was approved by the Local Ethics Committee and written informed consent was obtained from each patient.

Material and methods

The study involved 26 women [at the mean age of 24.6 years] admitted to the Department of Internal Medicine and Endocrinology of the Medical University of Warsaw. All women included into the study had to fulfill Rotterdam criteria of PCOS [4]. The exclusion criteria were: hyperprolactinemia, congenital adrenal hyperplasia, thyroid disease, other causes of amenorrhea such as premature ovarian failure, Cushing's syndrome and androgen-secreting tumor. None of the patients could become pregnant during the study. We excluded from the study group also people who have found deviations in proctologic, urological and orthopedic examination, specially including history and physical examination of the pelvis and lumbosacral spine.

Hirsutism was assessed according to the modified Ferriman-Galwey scale, where a score above 8 was considered as significant [22]. Diagnosis of polycystic ovaries using pelvic ultrasound examination was based on the presence of either 12 or more follicles (2–9 mm in diameter) or increased ovarian volume (>10 cm^3) [23]. No subject received medication at the time of the study.

All patients underwent clinical and hormonal assessments. At baseline (pre-Met), and after 3 months of the therapy with metformin at a dose of 500 mg given three times daily (post-Met). These included anthropometric measurements of height, weight, waist/hip ratio. The presence of acne and menstrual cycle frequency was also assessed. Amenorrhea was defined as absent menstrual bleeding in the past 90 days. Oligomenorrhea was defined as more than 35 days between cycles with fewer than eight menstrual periods in the past year. The blood tests were taken after an overnight fasting, between the third and sixth day of either a spontaneous or a progestagen-induced menstruation (menstrual bleeding was obtained after ten-day administration of dydrogesterone. Blood samples for endocrine measurements were obtained between 07.30 and 08.30 am. At the same time saliva was collected into glass vials. Serum and saliva samples were stored at –80ºC until assayed. Free testosterone (fT) and bioavailable testosterone (bioT) calculations were carried out according to the formula available on the website of the International Society for the Study of the Aging Male (ISSAM) (http://www.issam.ch/freetesto.htm) using the concentration of testosterone, SHBG and albumin measured in the same serum sample. The calculation method is described in details by Vermeulen [24]. Equilibrium dialysis was carried out to assess free testosterone (T EQ) and free androstenedione (A EQ) fraction using 96-well Eqilibrium Dialyser, Membrane MWCO 5kDa (Harvard Apparatus, USA) [25]. Radioactive testosterone (1,2,6,7,16,17-^{3}H-testosterone, PerkinElmer, Life and Analytical Sciences, USA) or androstenedione (1,2-^{3}H-androstenedione, American Radiolabelled Chemicals, Inc, USA) was used as a tracer. Tracers were thin-layer purified (HPTLC-Alu Silica Gel 60) according to manufacturer's instruction, using toluene:ethyl acetate 2:1, and used no longer than one month after purification. Serum was diluted 1:1 with 0.9% saline solution with 30 kBq/ml of tracer added. Samples were preincubated for 30 min at 37ºC with agitation. Immediately, 290 microliters of sample was transferred to the appropriate compartment of dialyser with opposite cell filled with 290 microliters of saline solution. Equilibrium was reached during dialysis within 20 hours at 37ºC with gentle agitation. Subsequently, radioactivity of samples from each compartment was measured. Free Androgen Index (FAI) was calculated as a ratio of the total testosterone concentration multiplied by 100 and divided by the SHBG level. Endocrine status was assessed by measurement of luteinizing hormone (LH), follicle-stimulating hormone (FSH), oestradiol, total testosterone (T), dehydroepiandrosterone sulfate (DHEA-S), thyrotrophic-stimulating hormone (TSH), and SHBG, using electrochemiluminescence immunoassays (Roche Diagnostics, Germany) and Elecsys 20l0 analyzer (Hitachi, Japan). Androstenedione (A) and 17OH-progesterone (17OH-P) concentrations were measured with chemiluminescence immunoassays (Siemens Healthcare Diagnostics Products Ltd, UK) using Immulite 2000. Salivary testosterone (salT) and androstenedione (salA) concentrations were measured using enzyme immunoassay kits (Salimetrics, USA).

Depending on distribution characteristics of the analyzed parameters, we employed either a t-test in cases of normal distribution or a Mann-Whitney test if distribution characteristics was not normal. All statistical analyses were performed using Statistica 9.0 software StatSoft. Data were presented as mean ±SD). Correlations were tested by analysis of covariance (ANOVA test) or Pearson rank correlation method, where appropriate. $P < 0.05$ was considered statistically significant.

Study was performed in the Department of Internal Medicine and Endocrinology in the Public Central Teaching Hospital, Medical University of Warsaw. The protocol was approved by the local ethics committee and written informed consent was obtained from each subject.

Results

The mean level of glucose in patients with PCOS before treatment was 86.7±7.6 mg%. After 3 months of metformin' treatment glucose concentration was 82.4±7.7mg%. The basal glucose concentration above 100 mg% was found in three patients before metformin treatment and in one patient after 3 months of therapy. All women (before metformin treatment) went two hours oral glucose tolerance test (OGTT). Mean concetration of glucose in OGTT was 95.6±27.9 mg%. Glucose level between 140 and 199 mg% was found in two women. Anthropometric characteristic of PCOS patients is shown in Table I. During 3 months of treatment with metformin, no significant changes in body weight or BMI were observed. The Ferriman-Galwey score didn't change significantly after the 3-month treatment period (mean 13.8 points). Androgen concentrations in PCOS women are shown in Table II (pre- and post metformin treatment), and percentage changes after metformin treatment are shown in Table III. The level of calculated free testosterone was about 5-6-fold lower than concentration measured in saliva, and 4-fold lower than assessed by use of equilibrium dialysis.

Very high correlation between salivary androstenedione and free serum androstenedione estimated by EQ ($p<0.01$ $r=0.85$) and total androstenedione in serum ($p<0.01$ $r=0.84$) has been shown in patient with PCOS before metformin treatment. After 3 months' therapy the correlation is still significant (respectively $p<0.05$ $r=0.77$, $p<0.05$ $r=0.66$). Correlation between T and T EQ, and A and A EQ in women with PCOS before and after metformin treatment was evident. Table IV shows correlation between salA/salT and A EQ, T EQ, which was respectively $p<0.01$ $r=0.73$ and $p<0.01$ $r=0.62$ in patients before metformin therapy. Analogous correlations were respectively $p<0.01$ $r=0.55$ and $p<0.01$ $r=0.31$ in patients after metformin therapy Table V. Correlations between salA/salT and total androgens in serum were respectively for T and A $p<0.01$ $r=0.64$ and $p<0.01$ $r=0.71$ in patients before metformin therapy and $p<0.01$ $r=0.70$ and $p<0.01$ $r=0.44$ in patients after the therapy.

Gastrointestinal adverse effects (reduced appetite, nausea) affected approximately two of the patients on metformin during the first 5 days of treatment.

Table I.
Characteristics of PCOS patients before [pre-Met] and after metformin therapy [post-Met], * - p.<0.01

	pre-Met (n=26)		post-Met (n=26)	
	mean±SD	median	mean±SD	median
BMI (kg/m²)	25.31±7.20	22.99	24.99±7.31	22.45
WHR	0.80±0.07	0.79	0.80±0.06	0.8
SBP (mmHg)	120.38±9.68	120	116.92±10.30	112.5
DBP (mmHg)	77.69±7.24	80	72.69±8.03 *	70
Insulin (mIU/l)	8.98±7.88	7.01	7.31±6.41	5.48

Table II.
Hormonal profile of PCOS patients before [pre-Met] and after metformin therapy [post-Met] * - p.<0.01

	pre-Met (n=26)		post-Met (n=26)	
	mean±SD	median	mean±SD	median
T (ng/ml)	0.74±0.39	0.71	0.60±0.37 *	0.47
sal T (pg/ml)	99.92±25.28	101.77	85.32±24.49 *	86.70
A (ng/ml)	4.89±2.02	4.2	3.69±1.28 *	3.2
sal A (pg/ml)	420.53±184.00	444.01	331.79±146.03 *	286.22
T EQ (pg/ml)	49.73±33.38	41.49	34.12±23.11 *	24.46
A EQ (pg/ml)	1326.42±530.04	1392.19	836.64±185.15 *	834.82
FAI	7.78±6.68	5.26	5.32±4.64 *	4.08
SHBG (nmol/l)	48.08±32.22	38.8	87.70±54.20 *	49.0
bioT (pg/ml)	302.26±199.89	263.4	217.36±174.61 *	167.0
fT (pg/ml)	12.14±8.08	10.03	8.64±6.62 *	7.04

Table III.
The changes in hormonal profile in PCOS women after 3 months of metformin therapy [%], Δ % = [postMet-preMet]/preMet x 100% , ↓ - decreased, ↑ - increased, * - p<0.01

	Δ (%) post - Met vs pre - Met (n=26)
ΔT (ng/ml)	↓ 18.1 *
Δ A (pg/ml)	↓ 24.56 *
Δ salT (pg/ml)	↓ 14.61 *
Δ salA (pg/ml)	↓ 21.1 *
Δ T EQ (pg/ml)	↓ 31.4 *
Δ A EQ (pg/ml)	↓ 36.93 *
Δ FAI	↓ 31.61 *
Δ fT (pg/ml)	↓ 28.83 *
Δ bioT (pg/ml)	↓ 28.09 *
Δ SHBG (nmol/l)	↑ 42.89 *

Discussion

The aim of the study was to determine the usefulness of salivary androgens as new diagnostic marker for diagnosis and monitoring of treatment of PCOS patients during metformin treatment. Metformin an insulin sensitizing agent is used for the treatment of women with polycystic ovary syndrome. Metformin has been shown to have beneficial effects on insulin resistance in nondiabetic women with PCOS [26,27,28,29]. It is suggested that metformin in PCOS indirectly reduces insulin level, dyslipidemia and systemic inflammation. However, recent placebo-studies failed to demonstrate its significant metabolic benefit [30]. Therapeutic benefits of metformin in PCOS include improving cardio-metabolic and reproductive abnormalities. Several clinical trials examined metformin effectiveness on lipids,

atherosclerosis and inflammatory markers, hormone levels, menstrual irregularities, ovulation induction, fertility, hirsutism, obesity parameters and quality of life in PCOS women [29,31-39]. That is why the evaluation of effectiveness of metformin treatment was not the aim of the study thus the comparison with other methods of treatment (such as oral contraceptive) was neglected. The last recommendation are that all patients with PCOS should be screened for impaired glucose tolerance (IGT) with a two hours oral glucose tolerance test. Patients with normal glucose tolerance should be rescreened at least once every 2 years, or more frequently if additional risk factors are identified. Those with IGT should be screened annually for development of type 2 DM. PCOS patients with IGT should be treated with intensive lifestyle modification and weight loss and considered for treatment with insulin-sensitizing agents. The potential role of insulin-sensitizing agents in the prevention of IGT and diabetes in PCOS was emphasized [40]. Dunaif et al. [41,42] demonstrated that women with PCOS are insulin resistant, independent of obesity. In our study metformin treatment resulted in a significant decrease in the concentration of androgens in serum and in saliva. The increase of SHBG level was also significant during metformin therapy. A number of studies indicate that metformin in PCOS women reduces testosterone levels, which can reduce symptoms of hyperandrogenism such as acne, hirsutism, abdominal obesity and amenorrhea [43,44]. Even in none-obese PCOS women, metformin has beneficial effect on metabolic parameters and hyperandrogenism [45]. The tissue-specific actions of metformin, as well as the molecular mechanisms involved in the liver, muscle, endothelium, and the ovary are elucidated [45,46].

About 30% of women with PCOS in our study were not recognized as hyperandrogenic based on total testosterone level. Serum analysis fails to identify biochemical hyperandrogenism in about 20–40% of patients with PCOS [20]. There is general agreement that total testosterone measurement alone leads to underestimation of number of normoandrogenic women. Samples used to estimate of serum levels of testosterone should be carried out early in the morning, before 9:00 am, in order to avoid the effect of the diurnal variation in testosterone production [47]. Recent studies have shown that the current methods of measurement of total testosterone are not sensitive enough for samples with very low testosterone concentrations, such as found in women, even in women with severe hyperandrogenism, but also in testosterone-deficient men or children [48-50]. However, sensi-

Table IV.
Correlation analysis of androgen status for the PCOS women before metformin therapy, NS – not significant

	T (ng/ml)	A (ng/ml)	salT (pg/ml)	salA (pg/ml)	T EQ (pg/ml)	A EQ (pg/ml)	FAI	fT (pg/ml)	bioT (pg/ml)
T (ng/ml)	x	r=0.80 P<0.01	NS	r=0.76 P<0.01	r=0.89 P<0.01	r=0.80 P<0.01	r=0.58 P<0.01	r=0.84 P<0.01	r=0.84 P<0.01
A (ng/ml)	r=0.80 P<0.01	x	NS	r=0.84 P<0.01	r=0.71 P<0.01	r=0.89 P<0.01	r=0.72 p<0.01	r=0.83 P<0.01	r=0.83 P<0.01
salT (pg/ml)	NS	NS	x	r=0.52 P<0.01	NS	r=0.37 P=0.05	NS	NS	NS
salA (pg/ml)	r=0.76 P<0.01	r=0.84 P<0.01	r=0.52 P<0.01	x	r=0.77 P<0.01	r=0.85 P<0.01	r=0.65 P<0.01	r=0.75 P<0.01	r=0.76 P<0.01
salA/salT	r=0.64 P<0,01	r=0,71 P<0,01	x	x	r=0.62 P<0,01	r=0.73 P<0,01	r=0.56 P<0,01	r=0.67 P<0,01	r=0.69 P<0,01
T EQ (pg/ml)	r=0.89 P<0.01	r=0.71 P<0.01	NS	r=0.77 P<0.01	x	r=0.75 P<0.01	r=0.88 P<0.01	r=0.93 P<0.01	r=0.93 P<0.01
A EQ (pg/ml)	r=0.80 P<0.01	r=0.89 P<0.01	r=0.37 P=0.05	r=0.85 P<0.01	r=0.75 P<0.01	x	r=0.77 P<0.01	r=0.78 P<0.01	r=0.79 P<0.01
fT (pg/ml)	r=0.84 P<0.01	r=0.83 P<0.01	NS	r=0.75 P<0.01	r=0.93 P<0.01	r=0.78 P<0.01	r=0.93 P<0.01	x	r=0.99 P<0.01
bioT (pg/ml)	r=0.84 P<0.01	r=0.83 P<0.01	NS	r=0.76 P<0.01	r=0.93 P<0.01	r=0.79 P<0.01	r=0.90 P<0.01	r=0.99 P<0.01	x

Table V.
Results from of correlation analyses of androgen status for the PCOS women after 3 months of metformin therapy, NS – not significant

	T (ng/ml)	A (ng/ml)	salT (pg/ml)	salA (pg/ml)	T EQ (pg/ml)	A EQ (pg/ml)	FAI	fT (pg/ml)	bioT (pg/ml)
T (ng/ml)	x	NS	NS	r=0.63 P<0.05	r=0.61 P<0.05	NS	r=0.42 P<0.05	r=0.61 P<0.05	r=0.64 P<0.05
A (ng/ml)	NS	x	r=0.45 P<0.05	r=0.66 P<0.05	r=0.47 P<0.05	r=0.75 P<0.05	r=0.62 P<0.05	r=0.60 P<0.05	r=0.59 P<0.05
salT (pg/ml)	NS	r=0.45 P<0.05	x	r=0.62 P<0.05	r=0.59 P<0.05	r=0.47 P<0.05	r=0.47 P<0.05	r=0.53 P<0.05	r=0.50 P<0.05
salA (pg/ml)	r=0.63 P<0.05	r=0.66 P<0.05	r=0.62 P<0.05	x	r=0.73 P<0.05	r=0.77 P<0.05	r=0.60 P<0.05	r=0.72 P<0.05	r=0.72 P<0.05
salA/salT	r=0.70 P<0.05	r=0.44 P<0.05	x	x	r=0.31 P<0.05	r=0.55 P<0.05	NS	NS	NS
T EQ (pg/ml)	r=0.61 P<0.05	r=0.47 P<0.05	r=0.59 P<0.05	r=0.73 P<0.05	x	r=0.45 P<0.05	r=0.44 P<0.05	r=0.95 P<0.05	r=0.95 P<0.05
A EQ (pg/ml)	NS	r=0.75 P<0.05	r=0.47 P<0.05	r=0.77 P<0.05	r=0.45 P<0.05	x	r=0.85 P<0.05	r=0.49 P<0.05	r=0.49 P<0.05
fT (pg/ml)	r=0.61 P<0.05	r=0.60 P<0.05	r=0.53 P<0.05	r=0.72 P<0.05	r=0.95 P<0.05	r=0.49 P<0.05	r=0.92 P<0.05	x	r=0.99 P<0.05
bioT (pg/ml)	r=0.64 P<0.05	r=0.59 P<0.05	r=0.50 P<0.05	r=0.72 P<0.05	r=0.95 P<0.05	r=0.49 P<0.05	r=0.90 P<0.05	r=0.99 P<0.05	x

tivity may be improved by methods based on mass spectrometry Currently there is increasing use of mass spectrometry methods to quantify steroid hormones. These methods become common practice for all steroid hormone measurements [51].

Therefore we have made an attempt to analyze other parameters characterizing laboratory androgen status and to examine the correlation between different ways of measuring the levels of androgens.

Testosterone circulates in blood mostly (98%) as bound to serum proteins, primarily to SHBG and albumin. Only about 1.5 % of serum testosterone is free [52]. Because SHBG binds testosterone with high affinity, SHBG-bound T is not the bioactive fraction – it means is not available in target tissues for action through androgen receptor [53]. In contrast, albumin binds testosterone with low affinity, and its dissociation is fast [54]. The sum of weakly albumin-bound testosterone and free testosterone is referred to as bioavailable for target tissues. Previous studies have evaluated the accuracy of free and bioavailable testosterone assays in men, but its usefulness in women turned out to be limited [55-58].

Free serum testosterone concentration in women is approximately 20-fold lower than free testosterone in men. Oestrogens stimulate SHBG production and may bind to SHBG with high affinity, thus SHBG level in women is highly variable and affects measurements of total testosterone [9]. In our study equilibrium dialysis experiments show that free serum fraction of androgens were highly decreased in PCOS women by metformin treatment. Free and bioavailable testosterone fractions were also reduced as a result of therapy in consequence of both total testosterone lowering and SHBG increasing. However, it is important to notice that the level of calculated free testosterone depends strongly on the accuracy of total testosterone assay used. Most immunoassays for testosterone were not designed or validated for the relatively low levels normally present in women (including women with PCOS) [9]. It is important to note that free testosterone measured by equilibrium dialysis or calculated requires a sensitive, specific, precise and accurate assay for total testosterone. Furthermore, the free testosterone analog-based assay should not be used in practice because of low compliance with reference method [59,60].

Salivary testosterone and androstendione tests offer a non-invasive estimation of bioavailable androgens. Steroid hormones are transported to the saliva on the basis of passive diffusion, proportional to its bioavailability (the ability of unbound and loosely bound fractions to pass throughout biological membranes).

The measurement of physiological biomarkers in whole saliva can provide a significant tool for assessing the immunological and endocrinological status associated with exercise and training [61]. Salivary testosterone measurements can be substantially influenced by the process of sample collection, are susceptible to interferences caused by the leakage of plasma into saliva, and dependent on the material used for the collection of saliva. There are gender differences in salivary testosterone levels and variance, the serum-saliva association, the relationship of salivary testosterone to age and pubertal development, and the stability of individual differences in salivary testosterone levels over time [62]. The assessment of salivary androgens has several practical advantages, e.g. non-invasive character, the possibility of multiple sampling, simplicity in use [63].

Salivary concentrations of steroids reflect the level of bioavailable steroids in serum, although concentrations may differ because of salivary gland metabolism. Intrinsic 17-ketooxidoreductase activity in the salivary gland had been demonstrated in vitro in several species and the main metabolite for testosterone is androstenedione [64-67].Thus testosterone level in saliva may not be identical with the unbound fraction circulating in plasma, because a part of the steroid hormone pool is metabolized by salivary glands [68,69]. However, the spontaneously occurring small transfer of 0.1% of the plasma proteins does not seem to affect substantially salivary steroid concentration [70]. Better correlation between salA vs T and A than salT vs T and A may be a confirmation of the significance of steroid hormones metabolism in salivary glands. In PCOS women before metformin treatment correlation between salA/salT and A EQ was higher ($p<0.01$ $r=0.73$) than correlation between salA/salT and T EQ ($p<0.01$ $r=0.62$). In women after metformin treatment correlation between salA/salT and A EQ, T EQ was significant ($p<0.05$ $r=0.55$, $r=0.31$ respectively). Additionally, Wellen et al. showed in the study of healthy women that the ratio of free androstenedione to free testosterone in plasma was about four-fold the salivary ratio between androstenedione and testosterone [71] which we confirm in this study.

Conclusions

We conclude that salT and salA may be indicators of hyperandrogenism in women and good diagnostic markers in monitoring the hyperandogenicity treatment using metformin in women with PCOS. Our results indicate that salA/salT ratio is a good representation of the clinical status of androgenicity, especially in patient with PCOS during metformin treatment.

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Founding Source

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