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Original Article

Salivary testosterone for the diagnosis of androgen deficiency in end-stage renal disease

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Abstract

Background. Hypogonadism is frequent in patients with end-stage renal disease (ESRD). Salivary testosterone (Sal-T) is a non-invasive tool to screen androgen deficiency in adult male with normal renal function. However, available data on its utility in ESRD are not conclusive.

Objectives. The objectives of the study were: (i) to compare free testosterone fractions in saliva (SAL-T) and serum (Free-T); (ii) to establish the correlation of Sal-T with circulating total (TT) and bioavailable testosterone (Bio-T); (iii) to detect androgen deficiency through Sal-T; (iv) to determine the correlation of Sal-T with clinical parameters.

Methods. The study included: 60 adult ESRD men on haemodialysis (20–60 years old) with decreased libido referred from two dialysis centres; 112 eugonadic and 40 hypogonadic adult men with normal renal function as controls. Simultaneous morning saliva and serum samples were obtained for testosterone measurements by liquid RIA (SAL-T; TT). Free-T and Bio-T were calculated by the Vermeulen equation.

Results. Sal-T (0.338 ± 0.177 nM) and Free-T (0.338 ± 0.165 nM) did not differ ($P > 0.900$) in ESRD as well as in control (0.337 ± 0.182 and 0.337 ± 0.172 nM, respectively; $P > 0.900$). Sal-T levels correlated positively ($P < 0.0001$) with Free-T ($r = 0.95$), TT ($r = 0.80$) and Bio-T ($r = 0.76$) in ESRD. Sal-T negatively correlated with age and years on dialytic therapy. Sal-T showed 100% sensitivity and specificity to differentiate patients with androgen deficiency (22%) from those with normal androgen levels (78%). Hypogonadism was hypergonadotrophic in 69% cases and hypogonadotrophic in 31%.

Conclusions. These data demonstrate the value of morning Sal-T testing as a non-invasive approach to screen androgen status in ESRD patients.

Keywords: end-stage renal disease; haemodialysis; male androgen deficiency; salivary testosterone; serum testosterone

Introduction

A number of metabolic and endocrine disorders linked to chronic uraemic syndrome remain a challenge in spite of the progresses in renal disease therapy [1,2]. As reported, two-thirds of men on haemodialysis (HD) have serum testosterone in the hypogonadal range [3,4]. This deficiency was ascribed to a combination of primary and secondary testicular failure [3]. Clinical features associated with hypogonadism include low libido, erectile dysfunction, mood changes, fatigue, decreased bone-mineral density, anaemia and loss of muscle mass and strength [5]. None of these signs and symptoms are specific of low androgen concentration but may raise suspicion [6]. Changes in prolactin, gonadotrophins and gonadal hormone levels are found in HD patients [7,8]; these alterations along with vascular, neurologic, psychogenic and other factors, such as medications, contribute to the development of sexual dysfunction [9].

In the general population, low levels of endogenous testosterone in men are related to higher risk of cardiovascular disease, depression and decreased survival [10]. Circulating testosterone is routinely measured in clinical laboratories for investigation of androgen-related disorders. In healthy adult men, testosterone circulates in plasma either free (2.23%) or bound to serum proteins [albumin: 49.9%, sex hormone binding globulin (SHBG): 44.3%, CBG: 3.56%] [11]. Bioavailable testosterone, including both the measurable free and albumin bound fractions, might reflect more accurately the clinical situation when total serum testosterone is borderline or steroid binding protein levels are impaired [12].

Saliva is a widely accepted sample source for steroid analysis and offers a non-invasive and stress-free alternative to plasma and serum [13]. It is particularly useful in anaemic and high-risk patients as those with end-stage renal disease (ESRD) [14]. Salivary testosterone (Sal-T) reflects the free fraction of plasma testosterone that is able to diffuse passively across the salivary glands, independently of salivary flow-rate [13,15]. A highly significant correlation between salivary and free testosterone (Free-T) was described in adult men [16–20]; also, SAL-T positively correlated with circulating total (TT) and bioavailable (Bio-T) testosterone in eugonadal and hypogonadal subjects with normal renal function [20]. Recently, Sal-T was suggested as a biomarker for the diagnosis of male androgen deficiency, representing an attractive alternative to plasma [13,18–20].

Early studies [21,22] assessed Sal-T in uraemic men, but data on its clinical utility were not conclusive. Since SHBG levels have been reported to be low, normal or increased in uraemic patients [23–25], measurements of the free steroid fraction in serum or saliva may become the most accurate approach. Unfortunately, the usefulness of SAL-T was not further investigated.

As in other countries, the population on haemodialysis in Argentina has been steadily increasing. Male ESRD with decreased libido are frequently referred to our department for endocrine evaluation. The aim of this study was to investigate if Sal-T could become a reliable non-invasive tool to screen the androgen status in these patients. For this purpose, the following steps were performed: (i) to compare free testosterone fractions in saliva (SAL-T) and serum (Free-T); (ii) to establish the correlation of Sal-T with circulating TT and Bio-T; (iii) to detect androgen deficiency through Sal-T; (iv) to determine the correlation of Sal-T with clinical parameters.

Materials and methods

Study population

Sixty male ESRD (aged 20–60 years old) who complained of decreased libido were evaluated. They were all on maintenance HD (three times a week) in two dialysis centres of the Buenos Aires metropolitan area. They had no evidence of active psychiatric disease, infection, uncontrolled congestive heart failure or acute complications from uraemia at the time of the study. Exclusion criteria were cognitive impairment and history of alcohol or substance abuse. Patients taking drugs that might affect testosterone concentrations (anabolics, glucocorticoids, estrogens, ketoconazole, spironolactone) or induce hyperprolactinaemia (haloperidol, metoclopramide, phenothiazine, chlorpromazine) within 3 months prior to the study were also excluded. Most patients were on drugs commonly used in ESRD (vitamins B, C, D supplementation, folic acid, phosphate and potassium binders). Other specific medications were: β-blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers (n = 21); human insulin (n = 16); subcutaneous recombinant human erythropoietin (rHuEpo) (n = 31; mean dose ± SD = 4355 ± 2882 U/week). Table 1 displays the clinical and biochemical findings in ESRD. Saliva samples were obtained after confirming the integrity of salivary gland function as previously described [20].

Androgen deficiency was established following the approach suggested by the Endocrine Society using a cut-off value of total testosterone <10.4 nM with Free-T ≤ 0.23 nM and Bio-T ≤ 5.2 nM in subjects with decreased libido [26,27]. Total testicular volume was determined using Prader's orchidometer (normal total volume ≥30.0 mL).

The reference population (C) consisted of 112 eugonadic subjects (aged 20–60 years old, BMI = 23 ± 2.0 kg/m^2) and 40 known hypogonadic subjects (n = 22, 25–70 years old, BMI = 24 ± 3.0 kg/m^2) with normal renal function. Patients treated with testosterone enantathe (250 mg every 2–3 week i.m.) were evaluated 1 month after the last injection.

Table 1. Clinical and biochemical data of 60 male patients on haemodialysis

Patient characteristic	
Age (median and range; years)	39.5 (18–60)
Body mass index (mean ± SD; kg/m^2)	23.8 ± 3.2
Married (n, %)	49 (80%)
Paternity (n, %)	20 (33%)
HDRT (mean ± SD; years)	5.7 ± 4.1
CRF aetiology	
Hypertension	35%
Diabetes mellitus	27%
Policystic renal syndrome	10%
Haemolytic uraemic syndrome	4%
Kidney stones	3%
Other	8%
Unknown	13%
Laboratory parameters (mean ± SD)	
Serum albumin (g/dL)	4.0 ± 0.2
Serum haemoglobin (g/dL)	11.0 ± 1.5
Serum SHBG (nM)	24.6 ± 9.9
Total serum calcium (mg/dL)	8.96 ± 1.06
Intact serum PTH (pg/mL)	512.0 ± 356.0
Kt/V	1.17 ± 0.27

HDRT, haemodialysis replacement therapy; CRF, chronic renal failure; the index Kt/V is a measure of dialysis adequacy, value of 1.2 was considered appropriately. Reference range values: BMI: 20.0–25.0 kg/m^2; albumin: 3.6–5.0 g/dL; haemoglobin: 12.0–14.0 g/dL; SHBG: 10.0–56.0 nM; calcium: 8.90–10.50 mg/dL; PTH: 10.0–70.0 pg/mL.

The protocol was approved by the Human Research Ethics Committee of the School of Medicine, University of Buenos Aires, Argentina, and all subjects gave informed consent to participate in the study.

Study design

Sample collection. All subjects obtained saliva in fasting conditions. They were instructed not to brush their teeth 2 h before the saliva collection. Subjects collected 3.5 mL of whole saliva by directly spitting into polypropylene tubes between 07.00 and 09.00 hours, and simultaneous blood samples were drawn in each case. In ESRD patients, samples were obtained before starting the second dialytic procedure of the week. All patients were normotensive, without peripheral edema, had normal serum albumin levels, and weight gained interdialysis did not exceed 2.5 L. To minimize preanalytical errors, saliva was discarded if contaminated with blood. In case of any possible pink coloration, a dipstick test was used to detect haemoglobin contamination. When necessary, a second saliva and blood sample were obtained 1 to 3 months after.

The supernatants of saliva and serum obtained after centrifugation (1000 g, 10 min) were kept at −20°C for hormone analysis.

Salivary testosterone

Testosterone in saliva was determined by an adapted ^{125}I double antibody test for the quantification of total testosterone in serum (DSL 4100, Diagnostic System Laboratories, Inc. Webster, TX) with the modifications previously described [20]. Aliquots of 200 µL of saliva samples were processed by duplicate following the described steps [20]. The sensitivity of the SAL-T assay was 3.47 pM. The intra-assay and inter-assay coefficients of variation (CVs) were <6.8% and <9.5%, respectively.

Blood assessments

Sex hormone binding globulin. SHBG, expressed as the maximal dihydrotestosterone (DHT) binding capacity, was performed using 50 µL of serum as described [20].

Serum Hormones. Serum total testosterone and estradiol levels were assessed by radioimmunoassay (DSL 4100 and DSL 4400, Diagnostic System Laboratories Inc, Webster, TX, respectively) following the manufacturer's guidelines. The analytical sensitivities were 0.28 and 0.017 nM, respectively. The intra- and inter-CVs were <6.0% and 9.0% for TT and <5.4% and 9.4% for estradiol. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL) levels were measured by coat-a count immunoradiometric assays (Diagnostic Products Corporation, Los Angeles, CA) with sensitivities of 0.15 mUI/mL, 0.10 mUI/mL and 0.1 ng/mL (2.12 mUI/mL), respectively. The intra- and inter-CVs were: <1.7% and <7.2% for LH, <3.8% and <5.9% for FSH and <3.8% and <5.9% for PRL.

Whenever hyperprolactinaemia was found, native and bioactive monomeric PRL were estimated. To assess monomeric PRL, equal volumes (200 µL) of a 25% (wt/vol) solution of polyethyleneglycol (PEG 6000 kD) and patient serum were mixed for 1 h at 4°C and centrifuged at 4500 rpm for 15 min. Immunoreactive PRL was measured in the supernatant and unprecipitated serum by RIA. A PRL recovery ≤40% was indicative of macroprolactinaemia, while the condition was unlikely to be present at values >60% [28].

Albumin

Albumin concentration was measured using a Technicon RA500 analyzer (Technicon Instruments Corporation, New York, USA) following the manufacturer's recommended protocol.

Other analytes included in Table 1 were performed by the clinical chemistry laboratories for patients' regular monitoring.

Circulating free testosterone and bioavailable testosterone concentrations

They were both calculated as described by Vermeulen [29] using a second-order equation based on SHBG, total testosterone and albumin concentrations [30].

Statistical analysis

Results are expressed as mean ± standard deviation unless otherwise specified. Correlations between serum and salivary steroid levels were evaluated by Spearman analysis. The receiver operating characteristic (ROC) curve was employed to graphically demonstrate the sensitivities and specificities of the different diagnostic tests and cut-off values optimized for sensitivities. Statistical analysis was performed with program Medcalc for Windows version 7.4.3.1. P-values <0.05 were considered statistically significant.

Results

Free testosterone levels in saliva and serum in ESRD and controls

In morning samples, Sal-T (0.338 ± 0.177 nM) and Free-T (0.338 ± 0.165 nM) did not differ (P > 0.900) in ESRD as shown in C (0.337 ± 0.182 and 0.337 ± 0.172 nM, respectively; P > 0.900). In addition, a positive and significant correlation (r = 0.95, 95% confidence interval r = 0.95–0.98, P = 0.0001) was found between Sal-T and Free-T in ESRD and in C (r = 0.97, 95% CI for r = 0.96–0.98, P = 0.0001) (Figure 1).

Correlation between SAL-T and serum testosterone concentrations in ESRD

TT and Bio-T were 12.94 ± 4.53 and 7.47 ± 2.96 nM, respectively. SAL-T correlated positively and significantly with both in ESRD (r = 0.80 and r = 0.76) as observed in C (r = 0.91 and r = 0.93); P < 0.0001 for all.

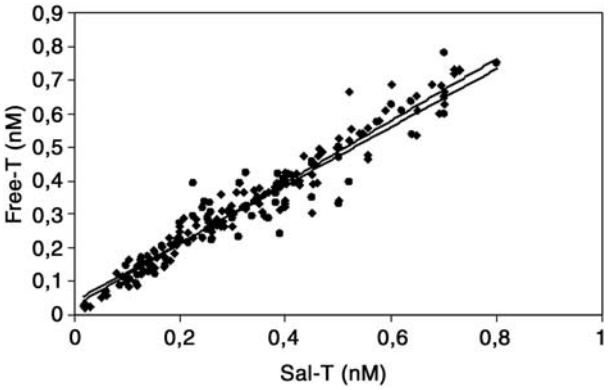

Fig. 1. Correlation between salivary (Sal-T) and serum free testosterone (Free-T) levels from simultaneously obtained morning samples in 60 male patients with end-stage renal disease and in 152 subjects without renal failure (reference group). Significant correlation (P = 0.0001) between Sal-T and Free-T was observed in ESRD patients (closed circles; r = 0.95) and in the reference group (closed diamonds; r = 0.97) constituted by 112 eugonadic and 40 hypogonadic subjects with normal renal function.

Salivary and serum testosterone levels in the reference group (C)

Androgen levels in eugonadic subjects were (mean ± SD): TT = 17.9 ± 5.3 nM, Bio-T = 9.32 ± 3.13 nM; Free-T = 0.410 ± 0.138 nM; Sal-T = 0.413 ± 0.148 nM. Hypogonadic subjects showed significantly lower androgen levels than eugonadic (P < 0.0001): TT = 6.14 ± 2.40 nM; Bio-T = 3.1 ± 1.43 nM, Free-T = 0.136 ± 0.06 nM, Sal-T = 0.134 ± 0.05 nM. ROC analysis defined TT ≤ 10.1 nM, Bio-T ≤5.3 nM, Free-T ≤0.229 nM and Sal-T ≤0.195 nM as the cut-off values with 100% sensitivity and specificity able to discriminate hypogonadic from eugonadic subjects [AUC_{ROC}: (95% CI) = 1.0 (0.976–1.00) in all cases].

Salivary and serum testosterone levels in ESRD: diagnosis of androgen deficiency

Figure 2 shows individual data of Sal-T, Free-T, Bio-T and TT in ESRD. In 47 ESRD, circulating testosterone fractions and SAL-T lay within the eugonadic range. Thirteen patients (22%) had Sal-T levels (0.129 ± 0.003 nM) in the hypogonadic range, confirmed by an additional second salivary sample (0.132 ± 0.003 nM, P = 0.792). Clinical and biochemical features of hypogonadal ESRD are shown in Table 2. High concentrations of LH and FSH were found in nine patients not associated to elevated monomeric PRL. In two ESRD with inappropriate gonadotrophin levels, high monomeric PRL concentrations were found.

Interestingly, a significant increase in mean serum LH (4.9 ± 1.1 mUI/mL) was observed in eugonadal ESRD compared to normogonadic C (2.4 ± 1.52 mUI/mL, P = 0.001) although lying within the normal range.

Serum estradiol in ESRD (eugonadic: 0.14 ± 0.04 nM and hypogonadic = 0.128 ± 0.05 nM) did not differ from C (0.15 ± 0.037 nM) P > 0.06 for all.

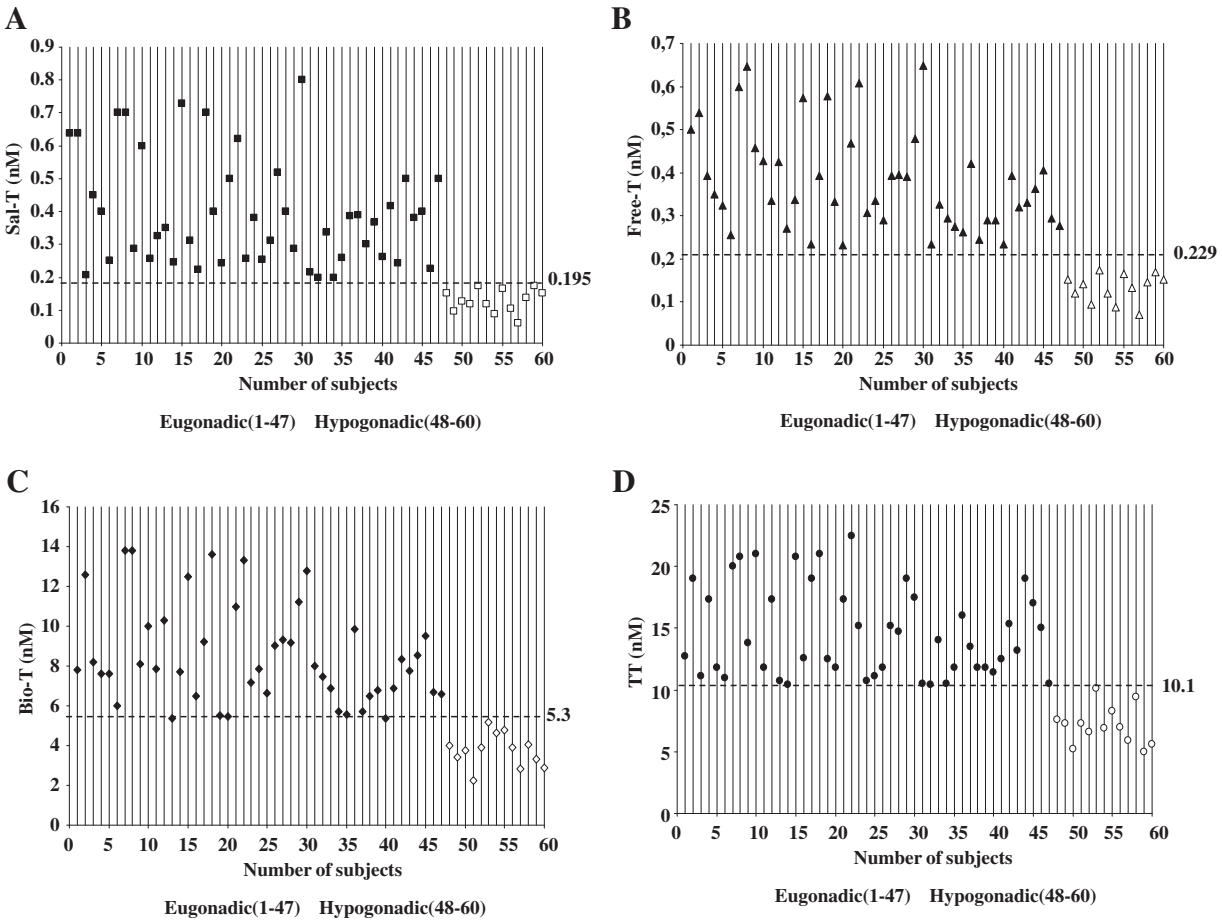

Fig. 2. Morning salivary testosterone (**A**), serum free testosterone (**B**), serum bioavailable testosterone (**C**) and serum total testosterone (**D**) levels in simultaneous samples obtained from eugonadic and hypogonadic male patients with end-stage renal disease. Dotted lines showed the cut-off values of SAL-T, Free-T, Bio-T and TT with 100% sensitivity and specificity able to discriminate hypogonadic ($n = 40$) from eugonadic subjects ($n = 112$) in the reference group.

Salivary and circulating testosterone in relation to clinical and biochemical parameters

Salivary testosterone correlated negatively with age in ESRD ($r = -0.312$, P = 0.012) (Figure 3) as in normogonadic C ($r = -0.465$, P = 0.001). Circulating TT, Bio-T and Free-T levels also correlated negatively with age in ESRD ($r = -0.261$; $r = -0.315$; $r = -0.383$, respectively; P ≤ 0.04 in all cases) and in normogonadic C ($r = -0.480$; $r = -0.454$; $r = -0.384$, respectively, P < 0.001 in all cases).

In ESRD patients, SAL-T had a weak negative correlation with number of years on dialytic therapy ($r = -0.270$, P = 0.038). Circulating TT, Bio-T and Free-T also correlated negatively with this variable ($r = -0.316$, $r = -0.311$ and $r = -0.358$, respectively, P < 0.02 for all).

Salivary and circulating testosterone in ESRD did not correlate significantly with either BMI or haemoglobin, albumin and parathormone levels. In addition, adequacy to dialysis (Kt/*V*) and rHuEpo dose administered did not show correlation with androgen levels. In rHuEpo-treated eugonadic patients ($n = 22$), TT (14.5 ± 3.0 nM), Bio-T (8.6 ± 2.0 nM), Free-T (0.381 ± 0.09 nM) and Sal-T (0.380 ± 0.157 nM) did not differ from those without rHuEpo ($n = 25$) (14.5 ± 4.2 nM, 8.43 ± 2.8 nM, 0.374 ± 0.137 nM, 0.428 ± 0.132 nM, respectively), P ≥ 0.260 in all cases. In nine hypogonadic patients treated with rHuEpo TT (7. 1 ± 1.5 nM), Bio-T (3.7 ± 1.0 nM), Free-T (0.157 ± 0.04 nM) and Sal-T (0.125 ± 0.04 nM) were not different from those in four patients who were not treated (7.05 ± 1.75, 3.9 ± 0.1, 0.173 ± 0.01 and 0.136 ± 0.03 nM, respectively), P ≥ 0.227 in all cases.

Discussion

This is the first study that demonstrates the value of Sal-T in the diagnosis of androgen deficiency in male ESRD. Morning Sal-T agreed with serum Free-T levels and correlates positively with all circulating testosterone fractions. Sal-T testing was able to differentiate patients with androgen deficiency (22%) from normogonadic patients (78%) with 100% sensitivity and specificity. Hypogonadism was hypergonadotrophic in 69% and hypogonadotrophic in 31%. Sal-T and all serum testosterone fractions correlated negatively with age and years on dialysis.

In the early 1980s, two studies described low TT levels in ESRD, but data on SAL-T where controversial [21,22]. Differences could be ascribed to the small number of pa-

Table 2. Clinical and biochemical findings in 13 hypogonadal patients on haemodialysis

Patient	Age (years)	Diagnosis	Paternity	Time (years)	TTV (mL)	TT (nM)	FreeT (nM)	Sal-T (nM)	LH (mUI/mL)	FSH (mUI//mL)	PRL (ng/mL)	PRL_{PEG} recovery (%)
48	29	HBP	No	7.0	24.0	7.6	0.152	0.149 ± 0.003	7.4	11.2	16.0	n.d.
49	29	UHS	No	15.0	16.0	7.3	0.120	0.099 ± 0.001	1.0	5.8	90.0	70.0[a]
50	36	PKD	No	6.0	14.0	5.2	0.141	0.131 ± 0.003	8.0	16.0	16.0	n.d
51	37	PKD	No	9.0	18.0	7.3	0.095	0.119 ± 0.001	7.5	9.5	39.7	47.0
52	39	HTA	No	8.0	20.0	6.6	0.174	0.166 ± 0.010	0.6	3.0	4.3	n.d.
53	46	Unknown	Yes	8.0	22.0	10.1	0.120	0.118 ± 0.002	11.0	13.0	16.0	n.d.
54	46	DM	Yes	2.0	25.0	6.9	0.087	0.092 ± 0.007	7.0	10.0	92.8	41.0
55	48	HTA	No	10.0	27.0	8.3	0.166	0.168 ± 0.003	10.0	5.0	20.0	n.d.
56	55	HTA	Yes	6.0	27.0	7.0	0.134	0.113 ± 0.012	16.0	10.0	5.0	n.d.
57	55	Unknown	No	2.0	16.0	5.9	0.070	0.064 ± 0.005	4.4	3.9	40.9	53.0
58	56	HTA	Yes	11.0	25.0	9.4	0.140	0.133 ± 0.008	9.0	4.0	12.0	n.d
59	59	HTA	Yes	13.0	12.0	5.0	0.170	0.179 ± 0.009	6.0	7.8	45.8	68.0[a]
60	60	DM	No	3.0	20.0	5.6	0.153	0.164 ± 0.015	30.0	17.0	6.0	n.d.
Refrence values (range)					30.0–50.0	10.4–33.0	0.23–0.73	0.200–0.729	0.5–6.0	1.0–9.3	1.8–16.0	

HBP, high blood pressure; UHS, uraemic haemolytic syndrome; PKD, policystic kidney disease; DM, diabetes mellitus; paternity, prior to haemodialysis replacement therapy; time, time elapsed since the first haemodialytic procedure; TTV, total testicular volume; TT, serum total testosterone; Free-T, serum free testosterone; Sal-T, salivary testosterone (mean ± SD) from two samples obtained in non-consecutive days; n.d., not determined. [a]$PRL_{PEG\ recovery}$,< 40% was indicative to macroprolactinaemia and >60% was indicative that hyperprolactinaemia is due to monomeric prolactin.

tients in both series, sampling (timing, collection device) or in the in-house radioimmunoassay methodology [21,22]. In this study, we proved in ESRD that Sal-T agrees with Free-T and correlates positively with all circulating testosterone fractions as described in healthy men [20,31]. The correlation between salivary and serum free steroid concentrations in our ESRD could be due to the normal protein levels of this population (SHBG and albumin). Testosterone levels in biological fluids may change with different methodologies, and troubles in the dosage may appear when levels are very low [27]. The Sal-T assay used in this study had a detection limit of 1 pg/mL in agreement with others [32,33], proving its utility when known hypogonadic adult men (reference group) where evaluated. Whenever SAL-T was low, a second saliva sample was obtained. The reproducibility of SAL-T levels in hypogonadic ESRD did not differ from other hypogonadic population [20].

Literature describes a high (26–56%) prevalence of hypogonadism in unselected males with chronic renal failure with TT levels ≤10.4 nM [2,4,21,22,34–36]. However, in younger ESRD patients with decreased libido, we found a lower percentage (22%) of androgen deficiency using similar criteria. In the remaining 78%, symptoms could be related to uraemia, chronic illness, diabetes, vascular disease, depression, medications, inadequate psychological and physical response to dialysis, etc. [37]. Interestingly, all patients who reported paternity had fathered before starting dialysis therapy.

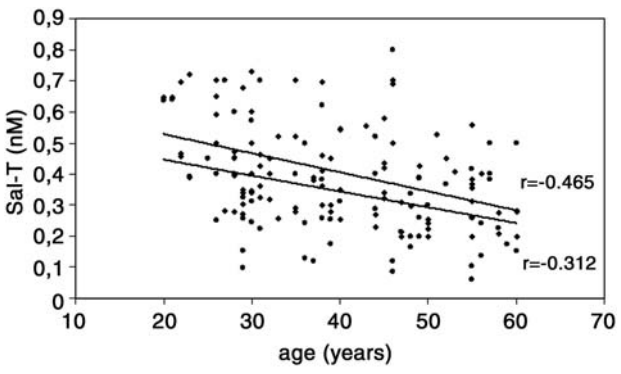

Fig. 3. Correlation between morning salivary testosterone levels (Sal-T) and age (years) in 60 male with end-stage renal disease and 112 eugonadic healthy subjects. Sal-T declines significantly with age ($r = -0.312$, $P = 0.012$) in HD (closed circles) as in 112 eugonadic healthy subjects (closed diamonds; $r = -0.465$, $P < 0.001$).

As previously described [38], high PRL levels were found in 10% of our patients; increased monomeric isoforms were confirmed in two hypogonadal ESRD patients. These patients started cabergoline, improving symptoms while decreasing PRL and normalizing androgens levels. As reported, this medical approach may benefit uraemic patients, although resistance and side effects to dopaminergic agents have been described [39]. Our next goal is to investigate benefits of testosterone therapy in hypogonadic ESRD [35].

As reported in healthy men, a negative correlation between circulating testosterone levels, SAL-T and age was confirmed in ESRD [4,36,40]. Anaemia and secondary hyperparathyroidism are frequent in ESRD. However, as reported by others [4], neither haemoglobin nor parathormone levels correlated with circulating and salivary testosterone concentrations. The treatment of anaemia with rHuEpo in male renal failure patients may affect sexual performance, although the mechanism of action remains controversial [41]. EPO stimulates Leidig cell steroidogenesis *in vitro* (rats [42]) and *in vivo* (healthy young males [43]). However, in agreement with Lawrence *et al.* [44], we could not confirm this effect in haemodialysis male patients. When focusing on the number of years on dialysis, a negative correlation was found with testosterone levels. Disorders of the pituitary–gonadal axis rarely normalize with dialysis; in fact, they often progress [7,39]. Since there is evidence that testosterone is not substantially cleared from circulation during haemodialysis [21,34],

hypogonadism in these patients seem to depend on reduced production of this hormone by the testes.

The assessment of SAL-T in ESRD met the criteria for diagnostic salivary steroid testing proposed recently [31]: there was a constant and predictable correlation between salivary and serum free steroid concentrations, the diagnostic accuracy of SAL-T was similar to that of serum testosterone, and a single saliva sample was just as informative as a single serum sample.

This study supports the usefulness of morning Sal-T testing as a non-invasive approach to screen androgen status in men with end-stage renal disease.

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Conflict of interest statement. None declared.

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