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From the author:

Teramoto and colleagues have completely misunderstood the purpose of the ERS Task Force on diagnosis and management of chronic cough [1]. The document deals with patients who have had a cough for >8 weeks. It is not about patients who can't cough. To suggest in their opening paragraph that we neglect cough in the elderly is simply disingenuous. We deliberately separated chronic cough in children from that in adults since the aetiology is different. However, in adults the causes and treatment of chronic cough are not age related and the elderly were frequent attendees in the 13 studies quoted in table 1 which presents the accumulated experience of specialist cough clinics [1].

Decreased cough and aspiration are important clinical problems but they were not the subject of our discussions. Clearly neurological illness [2, 3] and anatomical abnormality [4] can increase the likelihood of aspiration but this is neither age specific nor relevant to clinicians dealing with patients who present with isolated chronic cough.

Finally, an important function of documents such as the Task Force report is to provide a balanced overview of the literature. Teramoto and colleagues seem to have concentrated largely on their own work, which perhaps goes some way to explain the current debate.

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The hepatopulmonary syndrome: NO way out?

To the Editors:

The hepatopulmonary syndrome (HPS) is defined by the triad of chronic liver disease, abnormal pulmonary gas exchange (low arterial oxygen tension (P_{a,O_2}) and transfer factor of the lung for carbon monoxide), and intrapulmonary vascular dilatation [1]. The recent editorial on HPS [2] suggests that "hunting endogenous vasodilators that reduce pulmonary vascular tone logically became a sound strategy for those whose quest was to unravel the missing 'molecular' link between the diseased liver and the affected lung". But, is this strategy actually so logical? The key feature of the intrapulmonary vascular dilatation in HPS is the intrapulmonary shunt shown physiologically by a low P_{a,O_2} after 100% oxygen breathing, and anatomically by the passage of radiolabelled albumin macroaggregates (20–60 μm in diameter), or echobubbles, through the pulmonary capillary bed [3]. The striking feature pathologically is gross dilatation of capillaries in the alveolar septum, diameters of 100 μm, as compared with the normal 7–15 μm being described [4]. Is it likely that endogenous vasodilators are responsible for "relaxing" alveolar capillaries to such an extent? Of course, endogenous vasodilators may play a part in "remodelling" these capillaries.

With regard to pulmonary gas exchange, two factors seem to operate in severe hepatopulmonary syndrome: 1) a

Salivary 8-OHdG: A Useful Biomarker for Predicting Severe ED and Hypogonadism

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ABSTRACT

Introduction. Erectile and endothelial dysfunction are common pathologies of multiple cardiovascular risk factors and are considered longitudinal predictors of cardiovascular events. Oxidative stress and decreases in testosterone levels play an important role in the pathogenesis of endothelial dysfunction.

Aim. We sought to determine whether the severity of erectile dysfunction (ED) was associated with individual levels of testosterone and oxidative stress, and whether treatment with a phosphodiesterase type 5 inhibitor could reduce oxidative stress and increase testosterone availability.

Methods. We evaluated the association of salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG), salivary testosterone, International Index of Erectile Function-erectile function domain (IIEF-EF) scores, and MOS 36-item Short-Form Healthy Survey (SF-36) questionnaires in 128 middle-aged male volunteers. We investigated the changes in testosterone levels, salivary 8-OHdG levels, IIEF-EF scores, and SF-36 scores in 20 ED patients (according to the IIEF-EF) who took 50 mg of sildenafil once a week for 6 months.

Main Outcome Measures. IIEF-EF scores were used to assess ED severity. Antioxidant status was defined by salivary 8-OHdG. Salivary testosterone was used to evaluate serum bioavailable testosterone availability.

Results. Salivary 8-OHdG (OR = 9.88, 95% CI: 1.52–64.10), salivary testosterone (OR = 0.96, 95% CI: 0.93–0.98), and vitality on the SF-36, version 2 (SF-36 v2) (OR = 0.92, 95%CI: 0.84–0.98) were significantly associated with the severity of ED in healthy volunteers. Treatment with sildenafil for 6 months significantly increased the total serum testosterone (426.4 ± 174.8 vs. 569.6 ± 146.1 ng/dL, $P = 0.021$) and salivary testosterone levels (56.1 ± 22.3 vs. 110.0 ± 48.4 pg/mL, $P < 0.001$), whereas it decreased salivary 8-OHdG levels (2.30 ± 0.23 vs. 0.90 ± 0.05 ng/mL, $P = 0.0046$).

Conclusions. Salivary 8-OHdG is a useful biomarker for predicting severe ED and hypogonadism in middle-aged men. Once-a-week treatment with sildenafil can have beneficial effects on men's health by decreasing oxidative stress and increasing testosterone levels. **Yasuda M, Ide H, Furuya K, Takashi Y, Nishio K, Saito K, Isotani S, Kamiyama Y, Muto S, and Horie S. Salivary 8-OHdG: A useful biomarker for predicting severe ED and hypogonadism. J Sex Med **;**:**–**.**

Key Words. Erectile Dysfunction; Oxidative Stress; Salivary 8-OHdG; Salivary Testosterone; Sildenafil

Introduction

Erectile dysfunction (ED) profoundly affects the quality of life of both men and their partners [1]. Penile tumescence and erection rely on the release of nitric oxide (NO) by both cavernosal nerve terminals and endothelial cells [2]. The Massachusetts Male Aging Study showed that major cardiovascular risk factors are prevalent in individuals with ED [3]. Endothelial dysfunction arises after alteration in the release of several vasoactive factors, principally NO, from endothelial cells [4]. The pathogeneses of both endothelial dysfunction and ED are intimately linked through the physiological actions of NO [5]. The role of endothelial cells in the maintenance of penile erection underscores the close association of ED with endothelial dysfunction in

peripheral circulation [6] and with the presence of cardiovascular risk factors [7].

Evidence is accumulating that ED manifests the early onset of cardiovascular disease [8]. Oxidative stress is a major cause of endothelial dysfunction. Oxidative stress produces reactive oxygen species, mainly superoxide, and may promote endothelial dysfunction by inactivating the production of NO [9,10]. Oxidative stress might play a significant role in the pathophysiologic mechanism of ED [11]. Improving the balance of oxidative status to decrease oxidative stress could benefit therapeutic interventions and preventive strategies in restoring endothelial function and treating ED [12].

However, there are no previous clinical studies that examined the association between the extent of oxidative stress and the symptoms of ED.

Among all bases in nucleic acid, guanine is the most susceptible to oxidative damage and is oxidized to 8-hydroxy-2′-deoxyguanosine (8-OHdG). As it is stable and excreted in bodily fluids with DNA repair, 8-OHdG is one of the most commonly used markers for evaluating oxidative damage [13].

Testosterone maintains endothelial function by increasing the metabolism of the NO-mediated pathway [14]. Although it is a debated issue that low testosterone levels are associated with premature death in men [15], preliminary evidence shows that low testosterone levels cause a number of comorbid diseases including diabetes and metabolic syndrome [16].

Phosphodiesterase type 5 (PDE5) inhibitors revolutionized the management of patients with ED. Several studies have shown that PDE5 inhibitors improve coronary endothelial function in patients with ischemic heart disease and heart failure [17], and endothelial dysfunction caused by oxidative stress [18].

Molecular science of erection physiology posits that PDE5 serves an important biological role. Current research suggests that PDE5 biology in the penis is not static but rather is subject to various forms of modulation, and that the enzyme is an opportune pharmacotherapeutic target for preserving penile health by interventions such as exogenous testosterone replacement or pharmacologic optimization of NO signaling in the penis using PDE5 inhibitors [19].

Aim

We sought to investigate the association between the severity of ED and testosterone levels and oxidative stress in a cross-sectional occupational study. We further investigated whether the chronic treatment of ED patients with PDE5 inhibitors modulated the extent of oxidative stress and testosterone levels.

Methods

The institutional review board at Teikyo University approved this study, and all subjects provided a written informed consent.

We evaluated the association of salivary 8-OHdG, salivary testosterone, International Index of Erectile Function-erectile function domain (IIEF-EF) scores, and MOS 36-item Short-Form Healthy Survey (SF-36) questionnaires in 128 middle-aged male volunteers. We investigated the changes in testosterone levels, salivary 8-OHdG levels, IIEF-EF scores, and SF-36 scores in 20 ED patients (according to the IIEF-EF) who took 50 mg of sildenafil once a week for 6 months.

Main Outcome Measures

IIEF-EF scores were used to assess ED severity. Antioxidant status was defined by salivary 8-OHdG. Salivary testosterone was used to evaluate the serum bioavailable testosterone availability.

Protocol of the Studies

Protocol 1: A Cross-Sectional Occupational Study

One hundred twenty-eight male volunteers without periodontal disease (mean age ± SD: 40.0 years ± 8.52), all office workers in the Tokyo metropolitan area, were included in this study. IIEF-EF scores were used to assess the prevalence and severity of ED [20]. Lower urinary tract symptoms were evaluated using the International Prostate Symptom Score (IPSS) [21]. Concomitant major depression was diagnosed through the Mini-International Neuropsychiatric Interview [22]. We evaluated the health-related quality of life of the subjects using the SF-36, version 2 (SF-36 v2) [23]. Serum and salivary levels of 8-OHdG, salivary testosterone, and salivary cortisol were evaluated. We excluded volunteers who had periodontitis, as previous studies showed that periodontitis patients had higher levels of salivary 8-OHdG [24].

Protocol 2: Clinical Treatment with Sildenafil

Twenty patients with ED (mean age ± SD: 54.65 years ± 8.40) (defined as persistent inability

to attain and maintain an erection sufficient for satisfactory sexual activity) whose IIEF-EF scores of 16 or less (moderate to severe ED [20]) were studied. Inclusion criteria included married with stable sexual relations with a female partner for at least 6 months before the study, as well as no previous treatment for ED. Subjects with kidney disease, liver failure, coronary heart disease, peripheral or cerebrovascular disease, endocrine diseases, prostatic disease, and major psychiatric disorders, except depression, were excluded. Patients concomitantly treated with nitrates or with congestive heart failure or those who were prescribed medicines that might modulate testosterone metabolism were also excluded. All participants were subjected to a full review of their medical histories and general examinations. Patients who satisfied the inclusion criteria were instructed to regularly take 50 mg of sildenafil on the evening of the same day during the weekend (either Saturday or Sunday) for 6 months, and were asked to spend time together with their partners after taking sildenafil. Patients and their partners visited our ED clinic every 4 weeks, and were asked to complete the IIEF-EF and SF-36 v2 questionnaires. The salivary levels of 8-OHdG and testosterone and serum hormonal profiles were determined for all subjects at the time of enrollment and at the end of the study by using the same methods.

Collection of Saliva

The procedure for the collection of saliva is described in detail elsewhere [25]. The subjects were provided with two Bakelite test tubes to collect saliva twice daily between 9 AM and 9:30 AM. They were asked to avoid eating, brushing their teeth, and smoking at least 1 hour before saliva sampling, as testosterone levels in saliva have been shown to increase post-microinjury by brushing teeth [26]. They rinsed their mouths with tap water three times and waited for 5 minutes, then expectorated at least 1 mL of saliva directly into the collection vial. The 5-minute delay was added to prevent the rinse from diluting the salivary testosterone, as it is measured in concentration per volume units (e.g., pg/mL). Salivary samples were stored at −20°C for up to 1 month in a laboratory freezer until analyzed.

Collection of Serum

For the simultaneous collection of saliva and blood, blood was drawn immediately after saliva sampling to avoid an increase in 8-OHdG and testosterone levels by taking a blood sample, which may be a stressor. Blood samples were centrifuged to isolate serum, and the serum was stored at −70°C until analysis.

8-OHdG Assay

We initially evaluated the correlation of values of serum and salivary 8-OHdG in 36 volunteers in protocol 1. Both serum and salivary 8-OHdG levels were measured by enzyme-linked immunosorbent assay (ELISA) (8-OHdG check high sensitivity, Japan Institute for the Control of Aging, Fukuoka, Japan).

The saliva samples were centrifuged at $11{,}000 \times g$ for 30 minutes and were filtered using an ultrafilter (cutoff molecular weight 10 kDa) to exclude interfering substances. The sensitivity of the assay was 0.125 ng/mL. The intra- and inter-coefficients of variation (CVs) were 2.1% and 7.1%, respectively.

Serum Hormone Assay

Serum total testosterone was measured by a chemiluminescent immunoassay (Architect testosterone, Abbott Japan Co., Ltd., Tokyo, Japan). The sensitivity of the assay was 0.08 ng/mL. The intra- and inter-CVs were 4.5% and 8.0%, respectively.

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by an electrochemiluminescence immunoassay (Elecsys LH and Elecsys FSH, Roche Diagnostics, Mannheim, Germany). Serum cortisol was measured by a single-step nonextraction coated tube radioimmunoassay (IM1841, Immunotech, Praha, Czech Republic).

Salivary Hormone Assay

The measurement of the salivary testosterone is a disputed issue. The Endocrine Society recently published its viewpoint on measuring serum total testosterone and free testosterone [27]. The Society gives any argument against salivary testosterone measurements, although a recent study demonstrated that salivary concentration measured by using ^{125}I antibody test was a reliable alternative to serum free testosterone concentrations [28].

We recently reported [25] that salivary testosterone measured by liquid chromatography/mass spectrometry and ELISA are reliable substitutes for serum free or bioavailable testosterone calculated by using the international formula [29]. Saliva testosterone levels were measured by

an ELISA (DE-SLV 3013, Demeditec Diagnostics, Kiel, Germany). The sensitivity of this assay was 1.8 pg/mL. The intra- and inter-CVs were 7.07% and 5.85%, respectively.

Saliva cortisol levels were measured by ELISA (DE-SLV2930, Demeditec Diagnostics).

Statistics

SPSS (15.0 version) was used for statistical analysis (SPSS Inc., Chicago, IL, USA). The Pearson correlation was calculated for the association between two continuous variables, and a Spearman's test was used for the association between salivary 8-OHdG and the IIEF-EF score. A Chi-square test was used for comparisons of categorical variables. Unpaired two-sided Student's *t*-test was used for comparison of means of normally distributed parameters, while a Mann-Whitney *U*-test was used in all other cases. Binominal logistic regression analysis was performed to examine the association between the severity of ED and various covariates. A paired *t*-test was used in the case of data with a normal distribution, and a Wilcoxson signed ranks test was used in the other cases. A P value <0.05 was considered statistically significant.

Results

Association of Oxidative Stress, Testosterone Level, and Severity of ED in Middle-Aged Office Male Workers (Protocol 1)

A relatively good correlation was seen between salivary 8-OHdG and serum 8-OHdG levels in the initial 36 subjects of protocol 1 (Fig. 1). Based on this result, salivary 8-OHdG was considered to be a noninvasive, reliable substitute for serum 8-OHdG. The prevalence of ED (IIEF-EF score <26) was 43.7% (56 cases). Among these, 32.8% (42 cases) had mild ED (IIFE EF score 17–25), 3.9% (5 cases) had moderate ED (IIEF-EF score 11–16), and 7% (9 cases) had severe ED (IIEF-EF score 1–10). The demographics of the two groups, that is, moderate and severe ED (N = 14) and normal or mild ED (N = 114) are shown in Table 1. Subjects with moderate and severe ED were significantly older than those with normal or mild ED. Patients with moderate and severe ED were significantly more depressed. They were more likely to smoke and to have medical comorbidity. Salivary 8-OHdG levels were significantly higher in those with moderate and severe ED than those with normal or with mild ED. Salivary testosterone levels were significantly lower in those with moderate and severe ED than those with normal or mild ED. For the SF-36 v2 questionnaire, the subjects with moderate and severe ED tended to have lower vitality scores. Differences between the two groups did not achieve a statistical significance in body mass index, habit of regular exercise, medical comorbidity (hypertension, diabetes, hyperlipidemia, or cardiovascular diseases), IPSS severity, salivary cortisol levels, and other domains of SF-36.

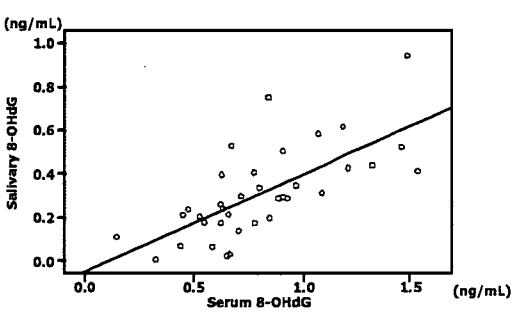

Figure 1 Correlation between salivary and serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels. There was a relatively good correlation between salivary and serum 8-OHdG levels (Pearson's correlation). N = 36, $r = 0.70$, $P < 0.001$, $y = 0.45x - 0.05$.

The associations between independent correlates and ED severity based on logistic regression analysis are shown in Table 2. The probability of having moderate and severe ED increased with salivary 8-OHdG, and decreased with both salivary testosterone and vitality on SF-36 v2. There was a weak inverse association of salivary testosterone and salivary 8-OHdG (Fig. 2). An inverse relationship was also evident between the IIEF-EF scores and salivary 8-OHdG (Fig. 3).

Effects of Clinical Treatment with Sildenafil on Oxidative Stress and Testosterone Levels in ED Patients (Protocol 2)

The baseline characteristics of ED patients who participated in protocol 2 and the changes of salivary 8-OHdG and hormone profiles, IIEF-EF scores, and SF-36 scores by chronic weekly treatment with sildenafil are shown in Table 3. Treatment with sildenafil significantly increased the IIEF-EF score, total serum testosterone levels, and salivary testosterone levels, while it significantly decreased salivary 8-OHdG levels. Changes of LH, FSH, and salivary cortisol levels did not achieve statistical significance.

Table 1 Characteristics of the 128 volunteers*

	Moderate and severe ED (N = 14, 10.9%)	Normal or mild ED (N = 114, 89.1%)	*P* value*
Demographics			
Age	48.5 ± 10.9	39.5 ± 8.20	<0.001
Physical status and lifestyle			
BMI, kg/m²†	23.0 ± 2.29	24.1 ± 2.77	0.15
Smoking habit (Brinkman's index)‡			
Median	140.0	133	0.047
(Percentile 25–75)	(11.3–413)	(0–325)	
Regular exercise, %§	21.4 (3 cases)	28.0 (32 cases)	0.74
Medical status			
Medical comorbidity, %¶	21.4 (3 cases)	12.2 (14 cases)	0.057
Depression, %**	21.4 (3 cases)	1.75 (2 cases)	0.002
IPSS severity††	1.00 ± 0.73	0.94 ± 0.59	0.74
IIEF-EF scores	9.46 ± 5.67	22.1 ± 2.37	<0.001
Biomarker			
Salivary testosterone (pg/mL)	47.3 ± 18.8	64.0 ± 26.7	0.025
Salivary 8-OHdG (ng/mL)	1.11 ± 0.76	0.54 ± 0.26	<0.001
Salivary cortisol (ng/mL)	0.21 ± 0.16	0.20 ± 0.16	0.42
Health-related quality of life (SF-36 v2)‡‡			
Physical health	51.1 ± 9.16	53.8 ± 6.08	0.14
Role-physical	52.6 ± 7.24	51.7 ± 8.39	0.71
Body pain	53.7 ± 9.44	53.5 ± 9.54	0.93
General health	48.9 ± 12.8	52.9 ± 8.62	0.14
Vitality	46.9 ± 9.99	51.7 ± 8.31	0.049
Social function	53.3 ± 7.60	51.9 ± 8.77	0.70
Role-emotional	54.8 ± 4.92	52.5 ± 7.70	0.24
Mental health	47.8 ± 8.13	51.3 ± 8.67	0.16

The data are presented as mean ± SD when normally distributed, while the data are presented as median (quartiles) when not parametric.
*The comparison was calculated with the use of Chi-square test for categorical variables. The comparison of means between two groups was calculated by unpaired two-sided Student's *t*-test for normal distribution and Mann–Whitney *U*-test for no normal distribution (smoking habit, regular exercise, medical comorbidity, and depression).
†Body mass index (BMI) is a measure of body fat based on height and weight, and calculated by the following formula. BMI = weight (kg)/height (m)².
‡Brinkman index means daily cigarette numbers multiplied by smoking years.
§Regular exercise refers to daily exercise such as a simple walk at a leisurely pace for 30 minutes.
¶Medical comorbidity is at least having one of four chronic diseases such as hypertension, diabetes, hyperlipidemia, or cardiovascular disease.
**Major depression was defined by the Mini-International Neuropsychiatric Interview, a short structured diagnostic interview developed jointly by psychiatrist and clinicians in the United States and Europe, for the Diagnostic and Statistic Manual of Mental Disorders, Fourth Edition (DSM-IV) and ICD-10 psychiatric disorder. Major depression is defined as the presence of five or more of nine symptoms such as depressed mood, loss of interest or pleasure, eating disorder, sleep disorder, psychomotor agitation, fatigue, a feeling of worthlessness or guilt, poor concentration, and suicidal ideation during the same 2-week period. At least one of the symptoms is either depressed mood or loss of interest or pleasure.
††Lower urinary tract symptoms were determined by using the International Prostate Symptom Score (IPSS), which consists of seven questions.
‡‡The SF-36, version 2 (SF-36 v2) was used to evaluate the subjects' health-related quality of life. The scores of each dimension are assigned a mean (±SD) score of 50 ± 10 on the basis of an assessment of the general Japanese population without chronic conditions. Individual scores were then compared with the normalized scores for the general population.
ED = erectile dysfunction; IIEF-EF = International Index of Erectile Function-erectile function domain; 8-OHdG = 8-hydroxy-2′-deoxyguanosine.

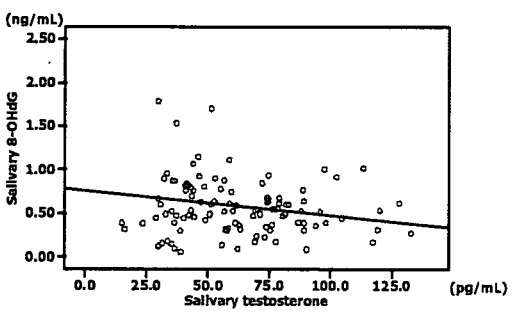

Figure 2 Association between salivary testosterone and salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels. There was a weak inverse association between salivary testosterone and 8-OHdG levels (Pearson's correlation). N = 128, $r = -0.21$, $P = 0.034$, $y = -0.003x + 0.766$.

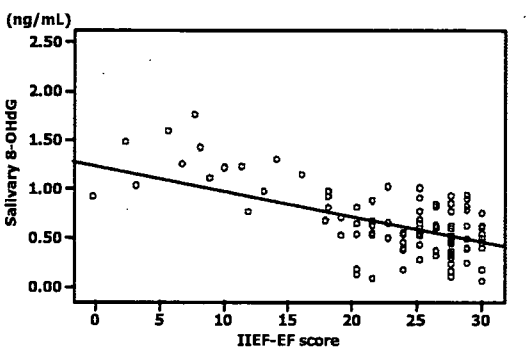

Figure 3 Association between the International Index of Erectile Function-erectile function domain (IIEF-EF) score and salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels. IIEF-EF scores significantly increased as salivary 8-OHdG levels decreased (Spearman's correlation). N = 128, $r = -0.33$, $P = 0.019$.

Table 2 Association between variables and ED severity: binominal logistic regression

Dependent variable Frequency of dependent variable	Moderate and severe ED (14/128) OR (95% CI), *P* value
Age	1.43 (1.03–1.43), $P = 0.17$
Smoking	1.01 (0.99–1.04), $P = 0.13$
Depression	0.27 (0.01–36.65), $P = 0.60$
Vitality on SF-36 v2	0.92 (0.84–0.98), $P = 0.048$
Salivary 8-OHdG	9.88 (1.52–64.10), $P = 0.016$
Salivary testosterone	0.96 (0.93–0.98), $P = 0.037$

ED = erectile dysfunction; SF-36 v2 = 36-item Short-Form Healthy Survey, version 2; 8-OHdG = 8-hydroxy-2'-deoxyguanosine.

Discussion

8-OHdG is most frequently measured as an indicator of generalized, cellular oxidative damage [13]. As there was a relatively good correlation between serum and salivary levels of 8-OHdG, we assumed that salivary 8-OHdG, a small-enough molecule to be secreted into saliva from serum through the salivary gland, could represent serum 8-OHdG. Our study showed that salivary 8-OHdG levels were independently and significantly associated with severe ED. The subjects with high salivary 8-OHdG levels were almost 10 times more likely to have moderate and severe ED. This result was consistent with previous studies indicating that oxidant stress may be central to both the acute and long-term pathophysiology of ED [30]. Although a PDE5 inhibitor has proved to be effective in the acute treatment of vasculogenic ED by preventing cyclic guanosine monophosphate (cGMP) degeneration leveling the cavernous smooth muscle cells, little consideration was paid to the preventative and curative approaches of ED in the long term. However, recent studies showed that chronic treatment with a PDE5 inhibitor could work as an antioxidant and improve endothelium-dependent vasodilatation in men [31]. A previous laboratory study in rats also showed that PDE5 inhibitors increased cerebral blood flow in the ischemic brain, plasma NO, cGMP, and total antioxidant status and attenuated endothelial dysfunction [32].

Our study showed for the first time that chronic treatment with sildenafil significantly improved the antioxidant status defined by salivary 8-OHdG. Free radicals and other reactive species cause oxidative damage to DNA that plays a crucial role in normal aging, and may contribute to pathologic processes associated with aging including arteriosclerosis, cancer, and neuron-degenerative disease. Oxidative DNA damage could be used as a biomarker to identify persons at risk of developing cancer and to suggest how diets of these persons could be modified to decrease that risk [33].

Furthermore, the 8-OHdG level is associated with occupational and lifestyle factors such as the length of working hours, number of cigarettes smoked, amount of alcohol consumed, and the scores of questionnaires for depressive mood [34,35]. Agents that decrease oxidative DNA damage are thus anticipated to decrease the risk of

Table 3 The change of hormone profiles, IIEF-EF score and SF-36 score by chronic treatment with sildenafil

Full sample, N = 20	Baseline	6 months	*P* value*
Age	54.65 ± 8.40		
LH (mIU/mL) median (Percentile 25–75)	4.30 (3.30–7.02)	4.90 (3.80–5.60)	0.53
FSH (mIU/mL) median (Percentile 25–75)	6.15 (4.00–8.01)	6.28 (5.10–7.90)	0.88
Cortisol (μg/dL)	15.4 ± 5.45	12.0 ± 5.52	0.10
Total testosterone (ng/mL)	426.4 ± 174.8	569.6 ± 146.1	0.021
Salivary testosterone (pg/mL)	56.1 ± 22.3	110.0 ± 48.4	$P < 0.001$
Salivary 8-OHdG (ng/mL)	2.30 ± 0.23	0.90 ± 0.05	0.0046
IIEF-EF score	8.40 ± 3.04	10.3 ± 5.03	0.049
Health related quality of life (SF-36 v2)			
Physical health	47.7 ± 7.16	50.4 ± 4.98	0.36
Role-physical	45.6 ± 11.6	48.0 ± 8.86	0.63
Body pain	49.9 ± 6.28	51.0 ± 7.47	0.75
General health	39.1 ± 12.8	44.0 ± 6.08	0.34
Vitality	38.2 ± 11.2	49.9 ± 10.4	0.06
Social function	48.9 ± 11.5	54.9 ± 4.65	0.17
Role-emotional	44.4 ± 9.75	47.6 ± 10.7	0.53
Mental health	48.5 ± 10.6	49.8 ± 6.10	0.75

The data are presented as mean ± SD when normal distributed, while the data are presented as median (quartiles) when not parametric.
*The comparison was calculated with the use of a paired *t*-test for normal distribution and Wilcoxson signed ranks test for no normal distribution (LH and FSH).
IIEF-EF = International Index of Erectile Function-erectile function domain; SF-36 = 36-item Short-Form Healthy Survey; LH = luteinizing hormone; FSH = follicle-stimulating hormone; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; SF-32 v2 = SF-36, version 2.

these age-related pathologies. We saw the long-lasting beneficial effects of sildenafil even though a significant inhibition of PDE5 by a 50 mg of sildenafil lasts around 6 hours. Previous studies show the beneficial effects of sildenafil on endothelial function and erectile function that outlast plasma exposure in both animals [36] and humans [37]. We hypothesize that behavioral modification as the effect of sildenafil might also be a factor in the change of oxidative stress and testosterone levels observed in this study [38].

The association between low testosterone levels and erectile function is arguable. A previous study showed a weak association between serum total testosterone and ED in elderly men [39]. The erectile function in elderly men often has a vascular or neurological origin [40,41] as well as endocrine origin. However, it has been demonstrated that testosterone controls the whole process of erectile function through nitric oxide synthase (NOS) production, PDE5 gene expression, and enzyme activity [42]. Therefore, even low testosterone levels, which affect NOS production and PDE5 activity, can initiate and maintain erectile function [43] by a sufficient amount of cGMP.

In our study, salivary testosterone levels were an independent predictor of severe ED. This inconsistency might result from the difference of the measurement of testosterone. Previous studies along this line used serum total testosterone. Previously, we showed that salivary testosterone can represent serum free and bioavailable testosterone [25].

Further research is warranted to investigate the best biomarker of testosterone availability in order to evaluate the association between testosterone levels and ED severity.

There was a slightly inverse association between oxidative stress and salivary testosterone levels in the volunteers (protocol 1). Furthermore, the treatment with sildenafil in the ED patients significantly increased testosterone levels and decreased 8-OHdG levels (protocol 2). These results indicate that oxidative stress, testosterone levels, and the severity of ED are associated. Individual testosterone levels tend to decrease gradually with age [44]. Several lines of evidence suggest that decreases in testosterone levels can affect men's health by promoting arteriosclerosis and mood disturbance, and decreasing insulin sensitivity [45]. Moreover, testosterone may play a role as an antioxidant in vascular injury by stimulating endothelial replication and inducing endothelium-dependent vascular relaxation [46].

Previous studies demonstrated that either sexual inactivity or few sexual stimuli damaged the reversible hypothalamic function, which decreased serum LH pulsatile secretion, and serum testosterone levels [47,48]. Our study found that regular sildenafil intake was likely to increase LH levels and significantly increase salivary testosterone levels.

There are also a number of studies consistent with a view that age-associated decline of endogenous testosterone may be a causal factor in cardiovascular disease and cancer [14,49]. Thus, maintaining proper testosterone levels is considered beneficial for men's health. To enlighten people that ED is not merely an issue in sexual life but also has other important health implications is essential. Biomarkers with easy access to screen the high risk of ED, such as salivary 8-OHdG and testosterone, can be helpful to promote this awareness. Further research is warranted to investigate whether a long-acting PDE5 inhibitor, such as tadalafil, is more effective to decrease oxidative stress and increase testosterone activity in the long term [50].

The limitation of this study is the small number of subjects in either protocol. Furthermore, the effect of sildenafil on oxidative stress and testosterone levels in ED patients should be tested in a placebo-controlled double-blind study. However, a placebo-controlled study was difficult to conduct in severe ED patient, as there was a concern that participants in a placebo arm might be discouraged and discontinue the study. Thus, this study should be considered as preliminary and exploratory.

ED is a unique pathological condition that men can detect by themselves, although a fraction of men actually consult physicians about their symptoms. Introducing a biomarker, such as 8-OHdG, that predicts the severe form of ED and hypogonadism to the regular checkups in the working places or in the community would promote men's health.

Conclusion

Salivary 8-OHdG was significantly associated with ED severity as a risk factor, whereas salivary testosterone was significantly associated as a protective factor. Salivary 8-OHdG and salivary testosterone levels can be considered useful biomarkers for screening for ED in middle-aged men. A long-term regular intake of sildenafil in patients with ED might improve their testosterone availability and oxidative status. Greater awareness and

treatment of oxidative stress, testosterone levels, and ED could not only prevent life-threatening cardiovascular events but also sustain the quality of life of men.

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Keywords

Salivary testosterone

Late-onset hypogonadism (LOH)

Liquid chromatography/ mass spectrometry (LC-MS)

Enzyme-linked immunosorbent assay (ELISA)

Diagnostic significance of salivary testosterone measurement revisited: using liquid chromatography/mass spectrometry and enzyme-linked immunosorbent assay

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Abstract

Background & Objectives: The use of saliva as a material for screening biomarkers has several advantages in the study of large research populations. Since testosterone is not bound to protein in saliva, salivary testosterone determination provides an excellent approach for the evaluation of serum bioavailable or free testosterone. Liquid chromatography/mass spectrometry (LC-MS) has been considered to be a gold standard for estimating serum total testosterone levels in male serum. Our objective was to evaluate the reliability of salivary testosterone levels as measured by LC-MS. We also investigated the association between salivary testosterone measured by LC-MS and that measured by enzyme-linked immunosorbent assay (ELISA) in order to evaluate the clinical application for ELISA measures.

Methods: The study involved 51 healthy male volunteers (median age = 57 years old; range = 30–85 years) and 29 patients with late-onset hypogonadism (LOH) (median age = 65 years; range = 55–78 years) in order to include a wide range of testosterone levels (median age of all subjects = 65 years; range = 30–85 years). Serum total testosterone was measured using LC-MS, and sex hormone binding globulin (SHBG) by immunoradiometric assay. Serum free testosterone and bioavailable testosterone levels were calculated using an international formula. Salivary testosterone levels were measured using LC-MS and ELISA.

Results: Salivary testosterone levels measured by LC-MS were in accordance with calculated serum free testosterone levels ($r = 0.655$, $p < 0.001$, $y = 0.91x+27.04$; where x is the salivary testosterone measured by LC-MS and y is the calculated free testosterone). Salivary testosterone measured by LC-MS and ELISA showed a strong correlation ($r = 0.808$, $p < 0.001$).

Conclusion: Salivary testosterone measured by LC-MS and ELISA is a non-invasive, reliable substitute for serum calculated free or bioavailable testosterone. Considering its cost advantage and technical

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convenience, ELISA for salivary testosterone is now recommended for the purpose of screening the androgen bioavailability level especially in a large population-based study.

Introduction

Interest and debate regarding late-onset hypogonadism (LOH), or testosterone deficiency syndrome are increasing throughout the world [1–5]. The Endocrine Society and the European Association of Urology define LOH as a clinical and biochemical syndrome associated with advanced age and characterized by physiological, psychological and sexual symptoms and a deficiency in serum testosterone levels [6,7]. To screen for and diagnose LOH, and to evaluate the efficacy of treatment, an accurate and reliable biochemical index is needed. A robust assessment of the accuracy of testosterone assays remains challenging [8]. Total testosterone circulates mostly in the blood and is 98% bound to serum proteins, primarily sex hormone binding globulin (SHBG) and albumin; only 1–2% of serum testosterone is free of bound protein [9]. The combination of albumin-bound (weakly bound) testosterone and free testosterone is referred to as bioavailable testosterone, which is available to target tissues for androgenic action [10,11]. Serum free and bioavailable testosterone levels calculated from measured total testosterone and SHBG, using the International Society for the Study of the Aging Male (ISSAM) formula are widely accepted [12]. Liquid chromatography/mass spectrometry (LC-MS) has been validated as a gold standard method for estimating serum testosterone levels in male serum using protocols specified by the Federal Drug Administration. It includes the determination of the limits of detection, the limit of quantification (LOQ), the characteristics of the calibration curve, and the within- and between-day reproducibility [13]. The direct measurement of free testosterone levels in serum is currently possible only by using radioimmunoassay with equilibrium dialysis methods, which cannot be used for routine analysis [6]. In addition, this procedure is time-consuming and expensive because at least two low testosterone values must be obtained to confirm the diagnosis of hypogonadism, due to intra-individual variation [14]. In addition, since testosterone levels in healthy men follow a circadian rhythm [15], blood collection should be done early in the morning, which is often difficult in outpatient services.

Testosterone, like all steroid hormones, exists in the saliva in the free (bioavailable) form. Measurement of salivary testosterone levels has a great advantage, overcoming the problems associated with serum testosterone measurements. Saliva collection is simple, non-invasive, and repeatable. The subjects collect their saliva at home at a particular time specified by the researchers. Salivary testosterone is neither bound to proteins nor conjugated [16]. Thus, measuring salivary testosterone does not require dialysis (separation according to the molecule size) before assaying. Previous studies have shown significant correlations between serum free and bioavailable testosterone and salivary testosterone levels measured using radioactive isotopes [17]. The aim of this study was to re-evaluate the use of salivary testosterone levels measured using LC-MS and enzyme-linked immunosorbent assay (ELISA) as a substitute for serum bioavailable or free testosterone levels for clinical practice.

Subjects and Methods

Subjects

This study was approved by the Institutional Review Board at Teikyo University. In order to measure a wide range of testosterone levels, participants included 29 newly diagnosed LOH patients (median age = 65 years; range = 55–78 years) whose serum total testosterone levels were less than 300 ng/dl, which is the generally acceptable lower limit of the normal testosterone range in healthy young men [7], and 51 healthy male volunteers (median age = 57 years; range = 30–85 years: median age of all patients = 65 years; range = 30–85 years).

In order to diagnose LOH, the aging males' symptoms rating scale (AMS), which was developed by Heinemann et al. [18] was utilized as well as serum total testosterone levels. The severity of LOH symptoms according to the

AMS total score was classified as none/little (17–26), mild (27–36), moderate (37–49), and severe (50 or more) [19]. At the time of the first visit, the median AMS score of the 29 newly diagnosed LOH patients in this study was 44 (range = 29–52).

All participants provided written informed consent.

Saliva collection

Subjects were provided with two bakelite test tubes in which to collect saliva twice between 9 am and 9:30 am on a single day. They were asked to avoid brushing their teeth and smoking for at least 1 hour before saliva sampling, as testosterone levels in saliva have been shown to increase post-micro-injury due to brushing teeth [20]. They rinsed their mouths with tap water three times and waited 5 mins, then expectorated at least 1 ml of saliva directly into a collection vial. The 5-min delay was added to prevent the rinse from diluting the salivary testosterone, as it is measured in concentration per volume units (e.g. pg/ml). Patients were asked to refrain from taking sugar-free chewing gum within 1 hour of sample collection, as this gum can change salivary testosterone results [18]. Salivary samples were stored at −20 °C for up to 1 month in laboratory freezers until analyzed.

Serum collection

For the simultaneous collection of saliva and blood, blood was drawn immediately after saliva sampling to avoid any increase in testosterone levels due to taking a blood sample, which may be a stressor. Blood samples were centrifuged to isolate serum, and serum was stored at −70 °C until analysis.

Hormone determination

Serum total testosterone and salivary testosterone levels were measured by LC-MS, as described elsewhere [21]. Serum samples (0.1 ml) and saliva samples (1 ml) were analyzed using an API4000 electrospray-ionization mass spectrometer (Applied Biosystems/MDS SCIEX, Ontario, Canada). An Agilent 1100 device (Agilent Technologies Inc, Santa Clara, California, USA) was used for high-performance liquid chromatography. An HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and a Cadenza CD-C18 column (3 μm, 3 × 150 mm; Imtakt Corporation, Kyoto, Japan) were used for the separation of steroids.

For the mobile phase, we used 0.1% formic acid solution (Solution A) and a mixture of acetonitrile and methanol (1:1, Solution B). Initially, a mixture of Solutions A and B at a ratio of 35:65 was used, and, subsequently, gradient setting was performed so that the volume of Solution B reached 100% within 1.5 mins. This state was maintained for 2.5 mins. After 0.01 mins, the solution was set so that the Solution A:Solution B ratio returned to 35:65 (first system), and this state was maintained for 3 mins. The flow rate was 0.35 ml/min, and the duration of the analysis was 8.5 mins. Measurements were performed in the positive ion mode, and the infusion volume was 10 μl. Measurements were performed under the following conditions: for the quantification of cortisol, we used a product ion m/z 327.1 (IS: m/z 331.3) produced from a precursor ion m/z 363.3 (IS: m/z 367.3). For the quantification of testosterone, we used a product ion m/z 97.3 (IS: m/z 97.3) produced from a precursor ion m/z 289.2 (IS: m/z 292.2). Ion spray voltage and ion source temperature were established as 5000 V and 500 °C, respectively. Serum at 100 μl was diluted with purified water to prepare a capacity of 1 ml. T-d3 (D/N/P, Toronto, Canada) was used as the internal standard methanol solution and its purity was 98.5%. Samples, 1 ml, of saliva and the diluted serum samples were added to 1 ng/100 μl of Td-3, and then stirred. Following the previous process, the samples were mixed with 4 ml of ethyl acetate, shaken for 10 mins, and centrifuged at 3,000 rpm for 10 mins. After the aqueous layer was frozen, ethyl acetate was isolated, and evaporated using a centrifugal evaporator. The extract was dissolved in 100 μl of 70% acetonitrile solution, and 10 μl of this solution was infused into an LC-MS device. SHBG was measured by immunoradiometric assay. Both serum free testosterone and bioavailable testosterone were calculated by measuring total testosterone, SHBG, and albumin concentrations and using the international formula [12]. Saliva testosterone levels were also measured by ELISA (DE-SLV3013, Demeditec Diagnostics, Kiel, Germany) [22]. Briefly, 0.5 ml of saliva sample was applied to the microtiter wells that were

Table 1 Features of healthy volunteers and LOH patients

	Healthy volunteers $N = 51$	LOH patients $N = 29$
Age (mean ± SD) (years)	56.75 ± 10.35	65.52 ± 7.7*
Serum albumin (mean ± SD) (g/dl)	4.21 ± 0.28	4.4 ± 0.16
Sex hormone binding globulin (mean ± SD) (nmol/l)	52.94 ± 27.14	48.28 ± 19.52
Serum total testosterone (mean ± SD) (ng/dl)	466.96 ± 147.70	238.71 ± 41.03***
Calculated free testosterone (mean ± SD) (pg/ml)	74.45 ± 21.02	40.27 ± 20.61***
Calculated bioavailable testosterone (mean ± SD) (ng/dl)	171.46 ± 51.26	96.93 ± 50.46***
Salivary testosterone (LC-MS) (mean ± SD) (pg/ml)	40.22 ± 16.90	33.00 ± 7.07*
Salivary testosterone (ELISA) (mean ± SD) (pg/ml)	42.85 ± 10.16	31.78 ± 10.65*

LOH, late onset hypogonadism; SD, standard deviation; LC-MS, liquid chromatography/mass spectrometry; ELISA, enzyme-linked immunosorbent assay. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

coated with a mouse monoclonal antibody directed toward an antigenic site on the testosterone molecule. Endogenous testosterone from a salivary sample competed with a testosterone–horseradish peroxidase conjugate for binding to the coated antibody.

Statistical analysis

SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis.

Statistical differences in each indicator between the healthy volunteers and patients with LOH were examined using a *t*-test. Pearson's correlation coefficient was used to examine the association between two valuables. A *p*-value of <0.05 was considered statistically significant.

Results

The mean age, serum testosterone, albumin, SHBG, calculated free and bioavailable testosterone and salivary testosterone, as measured by both LC-MS and ELISA in healthy volunteers and LOH patients, are shown in Table 1. There were statistically significant differences in age ($p = 0.012$), serum total testosterone ($p < 0.001$), calculated free ($p < 0.001$) and bioavailable tes-

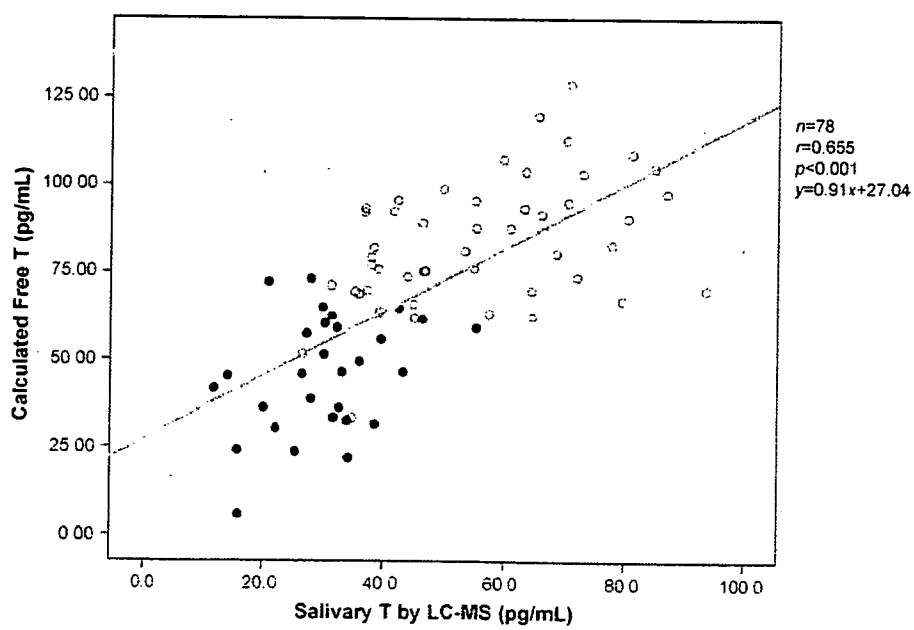

Figure 1 Significant correlation between salivary testosterone (T) as measured by liquid chromatography/mass spectrometry (LC-MS) and calculated serum free testosterone. Comparison of healthy volunteers (○) with LOH patients (●).

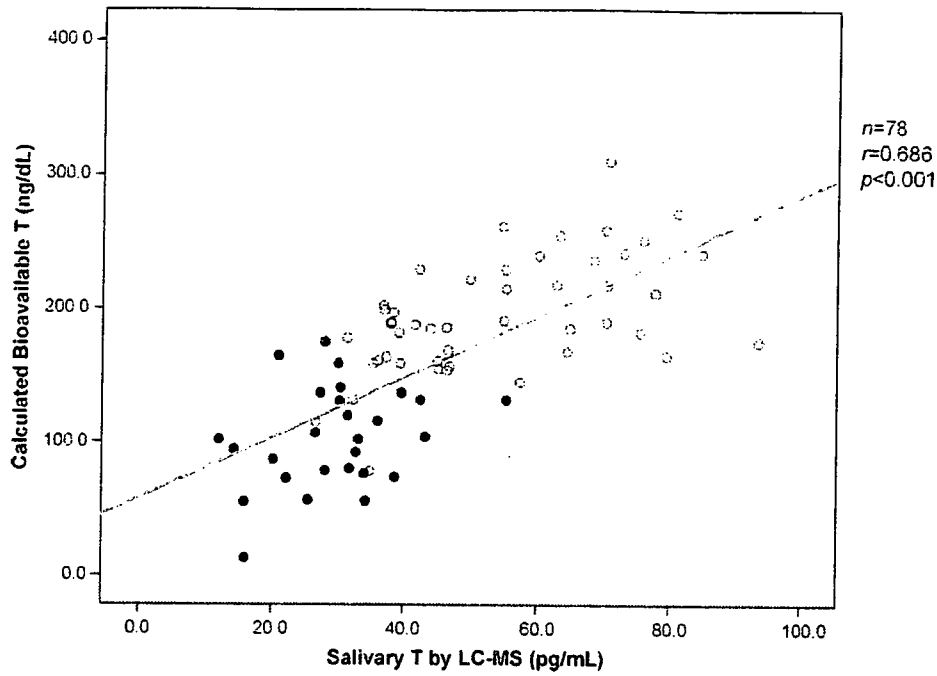

Figure 2 Significant correlation between salivary testosterone (T) as measured by liquid chromatography/mass spectrometry (LC-MS) and calculated bioavailable testosterone. Comparison of healthy volunteers (○) with LOH patients (●).

tosterone ($p < 0.001$) and in salivary testosterone as measured both by LC-MS ($p = 0.045$) and ELISA ($p = 0.048$) between the two groups. We confirmed that we could include subjects whose testosterone levels ranged from low to normal.

First, we examined the correlation between age and testosterone level and SHBG level. The

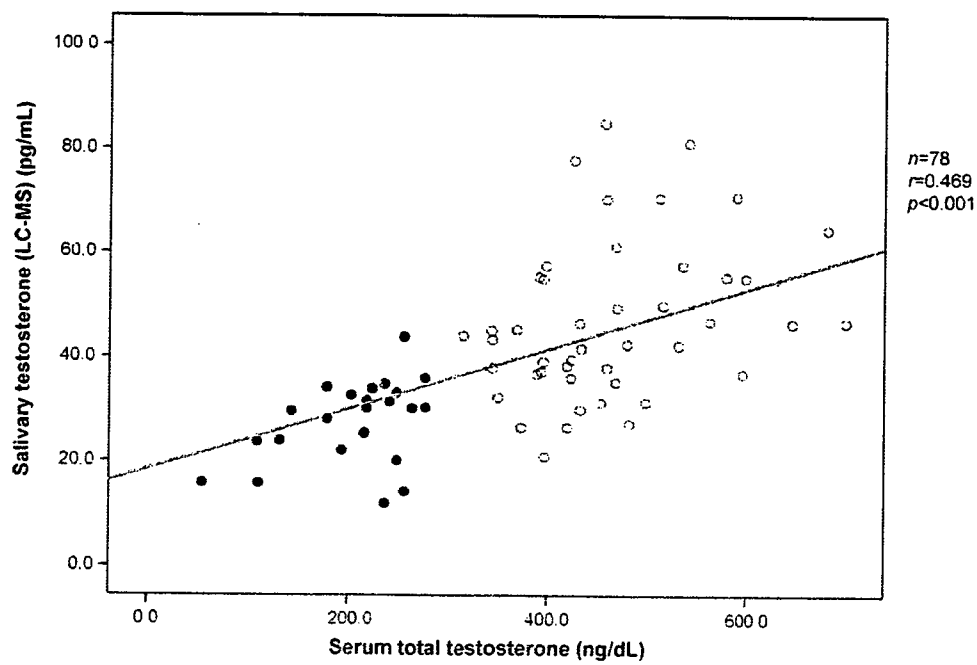

Figure 3 Correlations between salivary testosterone (T) and serum total testosterone. There was a parallel trend at high salivary testosterone levels, while a significant correlation was shown at lower testosterone levels. Comparison of healthy volunteers (○) with LOH patients (●).

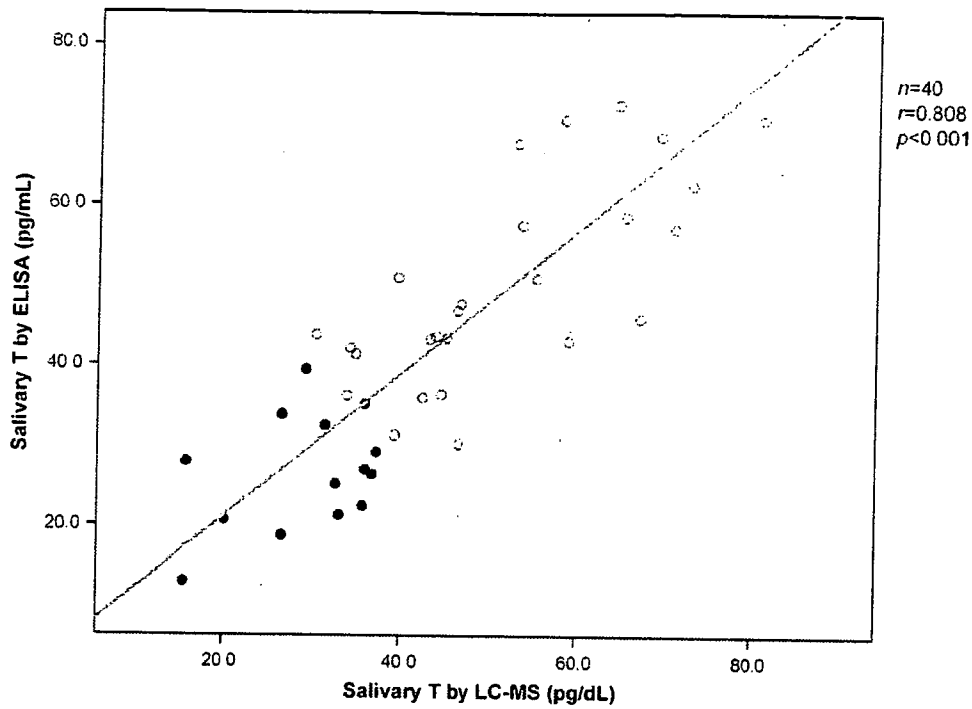

Figure 4 Strong correlation between salivary testosterone (T) as measured by liquid chromatography/mass spectrometry (LC-MS) and salivary testosterone as measured by enzyme-linked immunosorbent assay (ELISA). Comparison of healthy volunteers (○) with LOH patients (●).

serum total testosterone level measured by LC-MS was not significantly correlated with age ($p = 0.336$), whereas SHBG level significantly increased with age ($r = 0.338$, $p = 0.002$). Calculated serum free testosterone ($r = -0.387$, $p < 0.001$) and salivary testosterone as measured by LC-MS significantly, but slowly, decreased with age ($r = -0.381$, $p < 0.001$.)

Next, we examined the correlation between salivary testosterone as measured by LC-MS and serum testosterone. Salivary testosterone measured by LC-MS had a significant correlation with calculated serum free testosterone and both values were similar ($r = 0.655$, $p < 0.001$, $y = 0.91x+27.04$: where x is salivary testosterone as measured by LC-MS, and y is the calculated free testosterone; Fig. 1). Salivary testosterone as measured by LC-MS was significantly associated with calculated bioavailable testosterone ($r = 0.686$, $p < 0.001$; Fig. 2).

A significant correlation was shown between salivary testosterone level as measured by LC-MS and serum total testosterone ($r = 0.469$, $p < 0.001$; Fig. 3). The correlation between salivary testosterone levels as measured both by LC-MS and by ELISA was examined for the possibility of the clinical application of ELISA methods. A strong correlation was shown between salivary testosterone level measured by LC-MS and by ELISA ($r = 0.808$, $p < 0.001$; Fig. 4).

Discussion

Serum total testosterone levels are most commonly used to diagnose hypogonadism [6,7]. However, it should be noted that these testosterone values may not reflect the level of metabolically active testosterone. The most appropriate parameter for determining hypogonadism is probably the measurement of bioavailable testosterone [23]. In community studies, age-related declines in testosterone levels have been seen more frequently in bioavailable or free testosterone than in total testosterone [24,25] due to an increase in SHBG [23,25,26]. In our study, calculated serum free testosterone and salivary testosterone, as measured by LC-MS, significantly but slowly decreased with age. These observations were in accordance with previous studies [1,6,21]. It has been recommended that serum free testosterone level should be accurately measured using equilibrium dialysis or calculated from total testosterone, SHBG, and albu-

min concentrations using the international formula. Bioavailable testosterone levels can be measured using the ammonium sulfate precipitation method, the concanvalin A aggregation method, or it can be calculated in the same way as serum free testosterone using the international formula. However, these methods are time-consuming and costly for clinical practice. For example, measuring one SHBG level costs $150 in Japan, and this method is not covered by national health insurance.

In previous studies in the field of anthropology, salivary testosterone has been frequently adopted as an index for the assessment of examined subjects' androgen levels for several reasons [27–30]. Firstly, subjects feel more comfortable providing saliva rather than allowing blood to be drawn. Secondly, saliva can be collected without medical assistance. Thirdly, salivary testosterone is stable at room temperature for a few days before measurement. Fourthly, as testosterone is not bound to protein in saliva, measuring salivary testosterone does not require dialysis. However, in other studies, direct radioimmunoassay (RIA) was mainly used for the measurements of salivary testosterone [31]. In most of those previous studies, RIA had a good correlation for salivary testosterone with serum bioavailable and free testosterone [32]. Johnson et al. showed that $R = 0.83$ ($p < 0.001$) and Sannikka et al. showed $R = 0.75$ ($p < 0.001$) [33,34]. Vittek et al. also used direct RAI of salivary testosterone and found a strong correlation between serum free and salivary testosterone ($r = 0.97$, $p < 0.05$) [16]. However, Rey et al. performed a critical evaluation of free testosterone levels in plasma and saliva as measured by direct RIA. They failed to reveal a detectable testosterone concentration in saliva using the direct RIA method and concluded that salivary testosterone determined by this method did not reflect the true free testosterone values and that the significance of salivary testosterone remained to be established [35]. Although they agreed with the idea that salivary testosterone determination provided an excellent approach for the evaluation of androgen activity, they emphasized the discrepancy between salivary testosterone and serum free testosterone levels. They suggested that it might be due to the local conversion of androstenedinone to testosterone in the salivary glands or the possibility of the extraction of steroid-binding protein (such as SHBG) or albumin, which could bind free testosterone.

In addition to those contradictions, RIA is not a procedure that can be easily carried out in the laboratory nor is it an environmentally-friendly test. The advantages of the LC-MS for the measurement of salivary testosterone include easy and simple preparation (non-derivatized steroids can be directly analyzed), high recovery with improved signal-to-noise ratio, enhanced specificity, and low interference due to MS technology [36]. Moreover, it has been validated using protocols specified by the Federal Drug Administration and is considered to be a gold standard for determining testosterone levels. The superiority of the present study lies in the measurement of both serum total and salivary testosterone in order to investigate the association between salivary testosterone and serum testosterone bioavailability.

Notwithstanding, LC-MS is not an easy assay to perform, requiring numerous procedures. In measuring testosterone, ELISA is more cost effective ($5 per sample) and easier to perform than RIA and LC-MS (more than $40 per sample).

Travison et al. conducted a prospective cohort study with three data collection waves (1987–89, 1995–97, 2002–2004) and they reported that the past 20 years had seen a substantial age-independent population-level decrease in serum total and bioavailable testosterone. They concluded that the prevalence of low testosterone will exhibit an increase in excess of that expected given the projected aging of the population [37]. To screen biomarkers for evaluating health-related quality of life in community-based studies, sampling of salivary testosterone can be done anywhere in the community without medical help (e.g. at home for bedridden men) and make the examination of diurnal rhythms easy [38]. Based on our results, the values of salivary testosterone could be used as an alternative index to those of calculated serum testosterone without any need for complex calculations.

In conclusion, salivary testosterone is a non-invasive and reliable substitute for serum testosterone availability. Salivary testosterone level, as measured by ELISA, is recommended for use in large, population-based studies because of its clinical reliability, convenience and cost-effectiveness.

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Low testosterone level of middle-aged Japanese men – the association between low testosterone levels and quality-of-life

Keywords

Salivary testosterone

Middle-aged Japanese men

Quality of life

Late-onset hypogonadism

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Abstract

Background: Late-onset hypogonadism (LOH) is due to age-related steep declines in free testosterone levels in middle age. LOH can induce a variety of signs and symptoms that deteriorate the quality-of-life (QOL) of middle-aged men. This study examined the circadian rhythm of salivary testosterone levels in three age cohorts: 20s–30s, 40s–50s, and 60s+ to investigate the association between QOL and testosterone levels in adult Japanese men.

Subjects and Methods: Eighty-one healthy male Japanese volunteers and 20 LOH patients in their 40s–50s were recruited. Their salivary testosterone levels were measured as were independent variables including Body Mass Index (BMI) and smoking rates. The SF-36 v2 was used as the health-related questionnaire to evaluate QOL. Saliva samples were collected at 2-hour intervals between 9:00 am and 9:00 pm. Salivary testosterone levels were determined using an enzyme-linked immunosorbent assay (ELISA: Demeditec Diagnostics, Germany).

Results: There were no significant differences in BMI and smoking rates among the three healthy groups. However, scores from the SF-36 related to body pain were significantly lower in the 40s–50s cohort than in the 20s–30s group. The mean salivary testosterone levels in the 40s–50s group were the lowest at any point of time, except for 9:00 am among all healthy cohorts, and were similar to those of LOH patients. A circadian rhythm was seen in salivary testosterone levels in the 20s–30s and 40s–50s groups, whereas it was lost in the 60s+ group and in LOH patients.

Conclusion: Middle-aged Japanese men had the lowest levels of salivary testosterone and the worst QOL scores in relation to body pain, which may affect their overall QOL.

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Introduction

Promoting health and preventing disease requires a thorough understanding of the complex of social and behavioral factors that affect the health of individuals and the condition of communities.

Saliva has the advantage of allowing the measurement of biomarkers in a non-invasive and repeatable manner and this has created opportunities for behavioral scientists to test biosocial models of individual differences and intra-individual changes in mood, cognition, behavior and psychopathology [1].

The concept of age-related androgen deficiency in men, also termed late-onset hypogonadism (LOH), has opened up public awareness to the significance of men's health. Low testos-