Lower respiratory tract infection outcomes are predicted better by an age >80 years than by CURB-65

To the Editors:

In a recent issue of the *European Respiratory Journal*, Bont *et al.* [1] demonstrated that increasing age, previous hospitalisation, heart failure, diabetes, use of oral glucocorticoids, previous use of antibiotics, a diagnosis of pneumonia and an exacerbation of chronic obstructive pulmonary disease were independent predictors of 30-day hospitalisation or death in patients with lower respiratory tract infections (LRTI). They provided a new scoring system using the variables above for the prognostic predictors in the elderly primary-care patients with LRTI [1].

Although some of the predictor variables have been confirmed by other studies, we would like to point out that their results are very important, much more so than previous results.

Age is a well known risk factor for a poor outcome in LRTI. However, most related studies have recommended that an age ⩾65 yrs presents the greatest risk for a poor outcome of LRTI or community-acquired pneumonia (CAP) [2–4]. It has been recommended by the British Thoracic Society that a simple clinical prediction rule based on the five clinical features of age, confusion, urea, respiratory rate and blood pressure (the CURB-65 score) may be a practical means of stratifying patients with CAP into low-, intermediate- and high-mortality risk groups [4]. However, the study by Bont *et al.* [1] clearly indicated that an age >80 yrs presents the greatest risk for a poor outcome of LRTI.

We prospectively examined hospitalised pneumonia patients for 3 yrs (fig. 1). Most of the hospitalised pneumonia patients were ⩾65 yrs old. In fact, 75% of hospitalised patients with pneumonia were aged >70 yrs; therefore, an age ⩾65 yrs cannot be a meaningful cut-off level in terms of hospital admission risk and pneumonia risk. A similar phenomenon may occur in all developed countries, since the aged population is growing very rapidly; thus, in developed countries, CURB-65 may not be advantageous in the prediction of poor outcome in hospitalised LRTI or CAP. Unfortunately, most other studies in this area, which include a low number of elderly subjects, do not examine the significance of new age criteria, such as age ⩾80 yrs, being a better predictor for poor outcome than the conventional age criteria determined as age ⩾65 yrs.

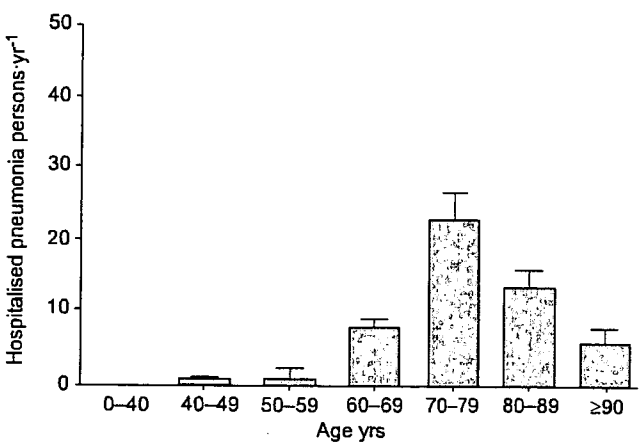

FIGURE 1. The mean±SD values of pneumonia incidence in the different ages for 3 yrs are presented.

It has been recently suggested that CURB-65 should not be supplanted by systemic inflammatory response syndrome (SIRS) or the standardised early warning score (SEWS) for initial prognostic assessment in CAP. Further research to identify better generic prognostic tools is required [5]. Although the SIRS and SEWS are different from LRTI and CAP, variables of age and pneumonia may be common contributors for the prognosis.

We would like to reinforce the fact that aspiration and silent aspiration are very important mechanisms of aspiration pneumonia in the elderly [6–9]. Since silent aspirations are very common in patients with stroke and frail elderly patients with advancing age, aspiration risk and dysphagia are significant predictors for the development of pneumonia and poor outcome of LRTI. Suspected aspiration is associated with more aggressive antibiotic treatment of suspected pneumonia episodes in nursing home residents dying with advanced dementia [10]

In conclusion, the current authors respect the fact that the CURB-65 score is useful to predict the outcome of patients with lower respiratory tract infections in the general population. However, the age cut-off point should be seriously reconsidered as significant as a good predictor for the outcome in the current clinical settings in aged populations of developed countries.

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STATEMENT OF INTEREST

None declared.

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DOI: 10.1183/09031936.00120807

From the authors:

We would like to thank S. Teramoto and co-workers for the important issues they raised. While appraising their comments, it is important to make a distinction between the use of severity rules inside and outside hospital settings. Looking at the available literature, we think that the pneumonia severity index (PSI) and CURB-65 (Confusion, Urea >7 mmol·L^{-1}, Respiratory rate $\geq$30 breaths·min^{-1}, Blood pressure (systolic value <90 mmHg or diastolic value $\leq$60 mmHg)) are both valid and useful in hospital settings. However, it is an interesting suggestion to improve CURB-65 by introducing more detailed age groups in the score. In primary care, PSI and CURB-65 are less useful for various reasons. Regarding the predictive value of age, the results of our study [1] showed that age >80 yrs was a better predictor of outcome than age categories between 65–80 yrs. Probably as there are a lot of healthy individuals aged 65–80 yrs in primary care who have a low risk for poor outcome.

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STATEMENT OF INTEREST

None declared.

REFERENCES

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DOI: 10.1183/09031936.00132907

Oral antibiotics prior to hospitalisation for community-acquired pneumonia

To the Editor:

SCHAAF *et al.* [1] postulate that antibiotics prior to hospitalisation with community-acquired pneumonia may be protective because of a slightly lower death rate and lower C-reactive protein concentration, leukocyte count and acute physiology score in the 13 out of 105 patients that received them. Since AUSTRIAN and GOLD [2] demonstrated a reduction in mortality from 80 to 17% in bacteraemic pneumococcal infections treated with penicillin, the death rate for this condition has changed little. A 2006 study has suggested that deaths in patients with community-acquired pneumonia are far more likely to be due to host factors rather than antibiotic choices [3].

It is possible that such host factors could lead to some patients having better outcomes, subacute presentations and more time before hospitalisation in which to receive oral antibiotics. Conversely, those patients with worse outcomes may show more acute presentations, removing the option of pre-hospitalisation antibiotics. Information on the number of days that patients were unwell prior to admission may help to answer this in part. Given the inaccuracy with which doctors make the diagnosis of community-acquired pneumonia, this is an important point [4–6], since pharmaceutical companies might be predicted to use potentially misleading conclusions such as this to encourage primary care physicians to prescribe antibiotics to anyone who might have community-acquired pneumonia, with potential for increased levels of antibiotic resistance, unnecessary costs and potential side-effects.

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STATEMENT OF INTEREST

None declared.

JGS	1597	B	Dispatch: 10.12.07	Journal: JGS	CE: Blackwell
Journal Name	Manuscript No.		Author Received:	No. of pages: 3	PE: Basha/prasath

LETTERS TO THE EDITOR

HIGH INCIDENCE OF ASPIRATION PNEUMONIA IN COMMUNITY- AND HOSPITAL-ACQUIRED PNEUMONIA IN HOSPITALIZED PATIENTS: A MULTICENTER, PROSPECTIVE STUDY IN JAPAN

To the Editor: Hospitalization for community-acquired pneumonia (CAP) in elderly people is associated with high mortality and with a high rate of readmission.[1,2] The recent data suggest that aspiration pneumonia due to silent aspiration is an important mechanism for the pathogenesis of pneumonia in older peoples.[3–5] Although the importance of aspiration as a frequent mechanism of CAP and hospital-acquired pneumonia (HAP), the incidence of aspiration pneumonia in hospitalized patients was not fully elucidated. Because the aged population is growing rapidly in developed countries, most hospitalized patients with pneumonia are older patients, who are likely to aspirate oropharyngeal contents during the night without witness.

We prospectively assessed the prevalence of aspiration pneumonia in CAP and HAP in hospitalized patients in 22 hospitals in different areas of Japan. Five hundred eighty-nine patients aged 2 to 101 (mean age ± standard deviation 72.6 ± 8.2; 377 men, 212 women) were studied between April 2004 and April 2005. Pulmonologists treated the hospitalized patients. No patients died in the hospital. In the current study, aspiration pneumonia was defined according to the Japanese Study Group on Aspiration Pulmonary Disease definition as pneumonia in a patient with a predisposition to aspiration because of dysphagia or swallowing disorders. Swallowing function was assessed using the water swallowing test, repetitive saliva swallowing test, simple-swallowing provocation test, and videofluorography.[6,7] The swallowing function testing was used for the diagnosis of aspiration pneumonia. When swallowing function was not assessed using these examinations, the presence of overt symptoms of dysphagia or the medical history of aspiration was determined as the swallowing disorders in the patients.

Pneumonia was diagnosed according to evidence of pulmonary infiltration examined using chest radiograph and computed tomography (CT) and according to systemic inflammation as determined according to blood analyses of white blood cell (WBC) count and C-reactive protein (CRP). The criteria for pneumonia were established according to the pneumonia guidelines of Japan Respiratory Society.[8]

Patients with pneumonia treated by another hospital before hospitalization in the study hospitals were excluded. Patients with severe complications and known allergies to the tested antibiotics, ands those who had received other antibiotic therapies within 1 month before enrollment were excluded. Patients with end-stage cancers or life-threatening serious disease or acute respiratory distress syndrome or sepsis were excluded from the study.

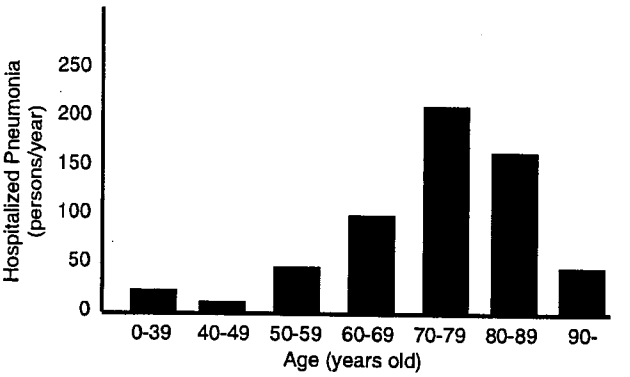

Figure 1. The incidence of hospitalized pneumonia according to age; 589 cases were analyzed.

In the current study, most of the patients hospitalized for pneumonia were elderly (Figure 1). Seventy-five percent of the hospitalized patients with pneumonia were aged 70 and older. The ratio of aspiration pneumonia to total cases of pneumonia increased with age (Figure 2). Aspiration pneumonia is common in patients aged 70 and older. Three hundred six of 382 pneumonia patients aged 70 and older (80.1%) were diagnosed with aspiration pneumonia.

The incidence of aspiration pneumonia in CAP and HAP was 60.1% (264/439 cases) and 86.7% (130/150 cases), respectively. Three hundred ninety-four patients of 589 patients hospitalized for pneumonia (66.8%) were diagnosed with aspiration pneumonia.

For the diagnosis of aspiration pneumonia, a swallowing function testing was performed on 361 patients (61.2%) in the current study. The water swallowing test was most frequently performed. Three hundred forty-four of 589 patients (58.4%) were examined using the water swallowing test. The repetitive saliva swallowing test and the simple-swallowing provocation test were used for approximately

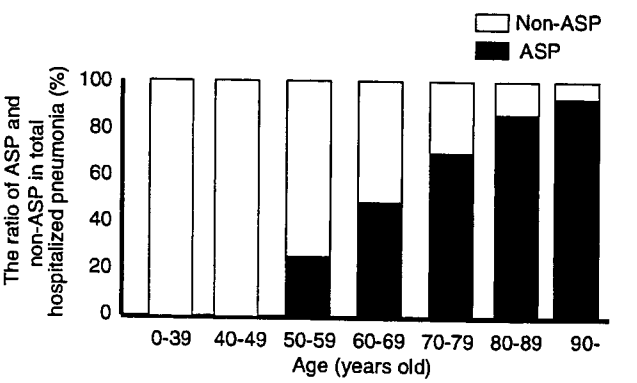

Figure 2. The ratio of aspiration pneumonia (ASP) and any type of pneumonia except aspiration pneumonia (non-ASP) in total hospitalized pneumonia according to age.

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20% of the patients. Only 6.2% of the patients with pneumonia were examined using videofluography.

This study revealed that aspiration pneumonia was common in CAP and HAP in hospitalized patients. Although the hospitalized patients were older, the incidence of aspiration pneumonia is high, which had not been previously speculated.

It has recently been reported that aspiration pneumonia is often observed in elderly patients with CAP.[5] Furthermore, the Perindopril Protection Against Recurrent Stroke Study (PROGRESS) found that angiotensin-converting enzyme inhibitors, which improve swallowing function, are effective in reducing the rate of aspiration pneumonia in Asian patients with a history of stroke but not in Caucasians.[9] Because the participants in the PROGRESS study were outpatients with a history of stroke but without overt neurological deficit, it is reasonable to speculate that silent aspiration and minor swallowing disorders after stroke cause aspiration pneumonia, and angiotensin-converting enzyme inhibitors induce improvement of swallowing function, which may contribute to a reduction in the frequency of pneumonia in otherwise healthy elderly subjects with a history of stroke. Because silent aspiration and swallowing dysfunction are frequently found in elderly patients without overt neuromuscular diseases and because hospitalized patients with pneumonia are mostly in their 60s and 70s, the incidence of aspiration pneumonia may be greater in future.

There are limitations of this study. Although it is a prospective multicenter study, the sample size was not large. Second, the swallowing function testing was not uniformly performed on the patients. Third, the study did not include bacterial examination. Furthermore, the pulmonary physicians in each hospital selected therapeutic regimens.

Although treatment with antibiotics is necessary to clinically cure CAP and HAP in elderly patients, recent data have revealed that oral care and swallowing rehabilitation are effective in preventing repeated aspiration pneumonia.[10] This evidence has suggested that dysphagia and swallowing abnormality may be primarily a mechanism of aspiration pneumonia in elderly people. Thus, in addition to antibiotic treatment, a preventive strategy based on aspiration-related mechanisms in the development of pneumonia may be important for hospitalized elderly patients.

In conclusion, the incidence of aspiration pneumonia as determined according to swallowing function testing was high in CAP and HAP in hospitalized patients. This indicates that the therapeutic and management approach for aspiration pneumonia may be necessary in the treatment guidelines for CAP and HAP in elderly people. Combined teams of geriatricians and infectious diseases and pulmonary specialists are likely to improve the quality of care in this situation. The high incidence of aspiration pneumonia also indicates that oral care and rehabilitation of swallowing disorders may be the key to prevention of CAP and HAP.

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ACKNOWLEDGMENTS

Conflict of Interest: The editor has reviewed the personal and financial checklist provided by the authors and has determined that none of the authors have any conflicts related to this letter. Dr. Teramoto was supported by a research grant from Mitsui Life Social Welfare Foundation Japan fund and by research grant from Mitsukoshi Health and Welfare Foundation in Japan.

Author Contributions: Shinji Teramoto: study concept and design. Yoshinosuke Fukuchi, Hidetada Sasaki, Koichi Sato, Kiyoihsa Sekizawa, Takeshi Matsuse: acquisition of subjects and/or data, analysis. Hidetada Sasaki and Kiyoihsa Sekizawa: interpretation of data. Shinji Teramoto: preparation of manuscript.

Sponsor's Role: Pfizer Japan Inc. supported the data collection and analysis. The sponsor had no role in the design, methods, recruitment, or preparation of this letter.

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JGS	1703	B	Dispatch: 3.3.08	Journal: JGS	CE: Blackwell
Journal Name	Manuscript No.		Author Received:	No. of pages: 2	PE: xx

LETTERS TO THE EDITOR

ANTIPLATELET CILOSTAZOL, AN INHIBITOR OF TYPE III PHOSPHODIESTERASE, IMPROVES SWALLOWING FUNCTION IN PATIENTS WITH A HISTORY OF STROKE

To the Editor: Stroke patients with dysphagia have a high incidence of aspiration, which may lead to pneumonia.[1] Several investigators have reported that cilostazol, a potent inhibitor of type III phosphodiesterase, reduces the incidence of aspiration pneumonia in patients with a history of stroke,[2,3] but the mechanism by which it does so has not been completely elucidated.

It has been proposed that swallowing disorders play a significant role in the development of pneumonia in patients with a history of stoke;[4,5] therefore, it was hypothesized that cilostazol may improve swallowing function in such patients by exerting pleiotropic effects through inhibiting type III phosphodiesterase. The effects of cilostazol administration on the serum and induced sputum levels of substance P, a transmitter in the nucleus of the solitary tract in the brainstem, were examined, because a significant association between systemic or airway depletion of substance P and silent aspiration and swallowing disorders has been reported in poststroke patients.[6,7]

The study subjects included patients with a 1- to 6-month history of noncardioembolic cerebral infarction confirmed using computed tomography or magnetic resonance imaging. In 48 male patients (mean age ± standard deviation 68 ± 5), swallowing function was examined before and after the administration of cilostazol and compared with swallowing function after the administration of aspirin. This study was a double-blinded, placebo-controlled, three-period crossover study wherein cilostazol administered at a dose of 200 mg twice daily and aspirin administered at a dose of 200 mg/d were compared with placebo. Patients were evaluated at baseline and after 4 weeks of treatment with each drug; a 4-week washout period was allowed after the completion of treatment with each drug. Swallowing function was examined using a repetitive saliva swallowing test and a swallowing provocation test.[4,5] In brief, the ability to swallow voluntary was quantitatively measured for 30 seconds. The swallowing reflex was induced by injecting a bolus of 1 mL of distilled water into the suprapharynx through a 5-Fr small nasal catheter with the patient in the supine position. The swallowing reflex was evaluated as the latency of the response.

The serum and induced sputum levels of substance P were measured using a radioimmunoassay.[8] After administering 5% saline solution via an ultrasonic nebulizer for 15 minutes, blood and induced sputum samples were collected between 7:30 and 8:00 a.m. before and after the completion of the cilostazol and aspirin treatments.

The significance of differences between the groups was assessed using analysis of variance, followed by *t*-tests with Bonferroni correction. The results are expressed as means ± standard deviations, and values of $P < .05$ were considered to indicate statistical significance.

Forty-eight patients completed the study. The latent time of swallowing reflex significantly improved after the administration of cilostazol but not after the administration of aspirin (Figure 1). Cilostazol increased the frequency of voluntary swallowing in 30 seconds (from 4.9 ± 0.2 to 5.6 ± 0.2), but aspirin did not (from 4.9 ± 0.2 to 5.1 ± 0.2). The induced sputum and serum substance P levels also improved after cilostazol administration sputum: from 16.6 ± 3.4 to 30.1 ± 6.2 pg/mL; serum: from 28.2 ± 4.4 to 44 ± 5.2 pg/mL) but not after aspirin administration (sputum: from 16.6 ± 3.8 to 18.1 ± 4.4 pg/mL; serum: from 28.2 ± 4.4 to 30.6 ± 4.6 pg/mL). No obvious untoward effects were observed.

The present study indicated that cilostazol but not aspirin improved swallowing reflex in patients with a history of stroke. Cilostazol also increased airway and systemic substance P levels in these patients. Thus, the improvement in swallowing function may contribute to the reduction in the incidence of pneumonia in poststroke patients treated with cilostazol.

Substance P is a transmitter in the nucleus of the solitary tract in the brainstem, and its level in induced sputum and blood is considerably associated with the swallowing function.[6,7] In experimental data, cilostazol has been reported to increase cyclic adenosine monophosphate (cAMP)-responsive element binding protein (CREB) phosphorylation, leading to upregulation of several apoptotic and dopaminergic genes.[9] This pathway may be involved in the improvement in swallowing function through the increase in substance P level in such patients.

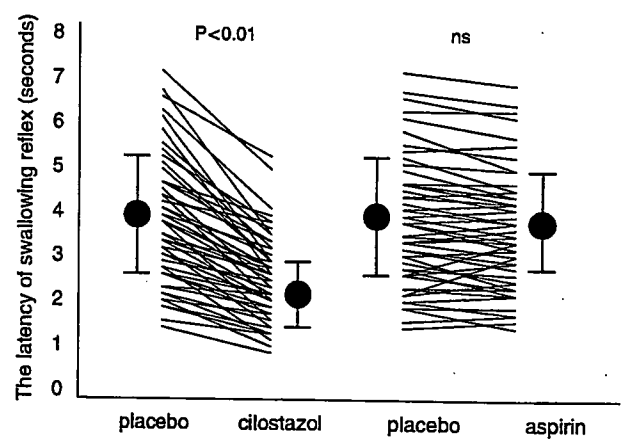

Figure 1. Effects of cilostazol and aspirin on swallowing reflex in patients with a history of stroke. The latency of swallowing reflex (seconds) was examined after 4 weeks administration of cilostazol, aspirin, and placebo. ns = nonsignificant.

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0002-8614/08/$15.00

The other mechanism underlying the improvement in swallowing function may be associated with saliva secretion, which the cAMP signaling pathway in the salivary acinar cells mediates.[10] The cilostazol-activated CREB plays a critical role in protein secretion from the parotid acinar cells. Increased saliva production by cilostazol may improve the swallowing function in these patients.

In conclusion, the potent inhibitor of type III phosphodiesterase—cilostazol—improves swallowing function through increased production of substance P in poststroke patients.

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ACKNOWLEDGMENTS

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the author and has determined that none of the authors have any financial or any other kind of personal conflicts with this letter.

Dr. Teramoto was supported by a research grant from Mitsui Life Social Welfare Foundation Japan fund and by research grant from Mitsukoshi Health and Welfare Foundation in Japan. There is no conflict of interest regarding the present study.

Author Contributions: Shinji Teramoto: study concept and design. Hiroshi Yamamoto, Yasuhiro Yamaguchi, Masaki Ishii, Hibi Shinichiro, Takeo Ishii: acquisition of subjects and/or data, analysis. Haruki Kume: interpretation of data. Yasuyoshi Ouchi: preparation of letter.

Sponsor's Role: No sponsor in this study.

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JGS	1689	B	Dispatch: 3.3.08	Journal: JGS	CE: Blackwell
Journal Name	Manuscript No.		Author Received:	No. of pages: 3	PE: Basha

LETTERS TO THE EDITOR

HIGH LEVELS OF CIRCULATING SOLUBLE CD40 LIGAND IN ELDERLY PATIENTS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME: EFFECTS OF CONTINUOUS POSITIVE AIRWAY PRESSURE

To the Editor: Patients with obstructive sleep apnea syndrome (OSAS) have a greater prevalence of coronary artery disease, myocardial infarction, nocturnal angina, and myocardial ischemia.[1–3] Although sleep apnea increases with age, the pathological role of OSAS has not been completely established in elderly people. One of the mechanisms implicated in vascular morbidity in adult patients with OSAS involves changes in nitric oxide pathways.[4,5] A soluble CD40 ligand (sCD40L) is an another marker for endothelium-related activation and dysfunction. This protein binds CD40 on the surface of various cell types of cells and triggers the increased expression of inflammatory mediators, growth factors, and the procoagulant tissue factor. The higher levels of sCD40L essentially indicate a greater risk of a variety of cardiovascular disorders. Furthermore, sCD40L levels were found to be higher in nonobese children with OSAS as well as adult patients with OSAS,[6,7] although circulating sCD40L concentrations and the therapeutic response of sCD40L to continuous positive airway pressure (CPAP) treatment have not been examined in elderly patients with OSAS.

We examined age-dependent differences in circulating levels of sCD40L in patients with OSAS. The experimental group consisted of 80 middle-aged (aged <60) and 80 elderly (60) patients with OSAS matched according to age and body mass index (BMI) to a control group of 80 middle-aged and 80 elderly subjects without OSAS. Subjects had to fulfill the following criteria: no renal and renovascular hypertension, systolic blood pressure (BP) less than 160 mmHg or diastolic BP less than 95 mmHg, no chronic renal or hepatic disease, and no diabetes mellitus. Patients with a history of smoking or systemic infections at the time of the study or in the 4 weeks before the study were excluded. No patients were taking antihypertensive agents. The subjects were examined using polysomnography (PSG) and classified as control subjects based on and apnea-hypopnea index (AHI) less than 5. In this study, to assess OSAS-induced hypoxia quantitatively, we used the oxyhemoglobin desaturation index (ODI) as previously described[4] (i.e., 90%). ODI was defined as $DI = \Sigma(90 - \text{oxygen saturation})t$, with t representing the time of desaturation in hours. Circulating sCD40L levels and sleep study variables were compared. Levels of circulating high-sensitivity C-reactive protein (hsCRP), which is a useful inflammatory marker in OSAS, were also examined as previously described.[8,9] The correlations between various parameters of OSAS, including ODI and oxidative stress, were also evaluated. Fasting blood samples were drawn using venipuncture in the morning between 7:30 a.m. and 8:00 a.m., after the PSG examination, into ethylenediaminetetraacetic acid—containing tubes. Blood samples were immediately centrifuged and frozen at −80°C until assay. Plasma levels of sCD40L were assayed with a commercially available enzyme-lined immunosorbent assay kit (BMS 235, Bender MedSystems GmbH, Vienna, Austria) with a sensitivity of 7.92 pg/mL. The intra-assay and interassay coefficients of variation were 6.4% and 5%, respectively.

There were no significant differences in BMI between patients and control subjects in each age group, although the AHI in the OSAS group was markedly higher than in the control group. There were no significant differences in BP and metabolic indices. AHI values in the elderly OSAS (50.1 ± 3.2 events/h) and middle-aged OSAS (51.6 ± 3.0 events/h) patients were considerably higher than for the age-matched controls (3.8 ± 0.3 and 3.6 ± 0.4 events/h, respectively). There were significant differences in baseline ODI values between OSAS patients and controls, suggesting that OSAS patients were exposed to a significantly greater degree of hypoxia than the control subjects. There were no differences in the ODI between middle-aged and elderly OSAS patients.

sCD40L levels in blood during the early morning hours in middle-aged and elderly OSAS patients were significantly higher than in their age- and BMI-matched controls, although age did not affect circulating sCD40L levels in OSAS patients. The values of hsCRP levels in middle-aged and elderly OSAS patients were significantly greater than those in controls, although there was no significant increase in hsCRP levels in elderly OSAS patients (Table 1).

A positive relationship was noted between circulating sCD40L levels and AHI or the magnitude of arterial oxygen desaturation, as indicated by ODI. This significant correlation was greater between circulating sCD40L levels and hypoxic episodes (ODI) (correlation coefficient (r) = 0.346, $P < .01$) than between circulating sCD40L levels and apnea episodes (AHI) ($r = 0.226$, $P < .05$). CPAP therapy significantly reduced circulating sCD40L in all patient groups.

These results indicate that the systemic inflammation and endothelial dysfunction indicated by high levels of sCD40L were observed in elderly patients with OSAS. Because sCD40L levels were significantly correlated with severity of hypoxia, as indexed according to ODI, the greater oxidative stress resulting from the considerable hypoxic stress rather than the apnea episode itself was in part responsible for the increase in sCD40L levels in elderly patients with OSAS. The improvement of sCD40L levels after treatment with CPAP may be consistent with the reduction in OSAS-related oxidative stress. Age-dependent differences in sCD40L levels and the therapeutic response of sCD40L to CPAP treatment were not observed in patients with OSAS. Similar results were observed in circulating adrenomedullin levels in elderly patients with OSAS,[10] although hsCRP levels in elderly patients with OSAS were

JAGS 2008

0002-8614/08/$15.00

Table 1. Circulating Soluble CD40 Ligand Levels and Other Variables in Elderly and Middle-Aged Subjects with Obstructive Sleep Apnea Syndrome (OSAS) and Controls

Characteristic	Middle-Aged OSAS Group (n = 80, 40 Male, 40 Female)	Elderly OSAS Group (n = 80, 40 Male, 40 Female)	Middle-Aged Control Group (n = 80, 40 Male, 40 Female)	Elderly Control Group (n = 80, 40 Male, 40 Female)
	Mean ± Standard Deviation			
Age	46.8 ± 2.2	65.8 ± 2.2	45.1 ± 2.2	64.8 ± 2.1
Body mass index	33.4 ± 0.9	32.1 ± 0.9	32.8 ± 1.1	31.5 ± 1.1
Systolic blood pressure	138.1 ± 3.7	139.1 ± 4.7	135.6 ± 4.1	137.1 ± 3.7
Diastolic blood pressure	82.1 ± 3.2	80.1 ± 3.2	78.9 ± 3.8	78.1 ± 2.8
Total cholesterol, mg/dL	202.9 ± 7.9	199.1 ± 6.9	202.9 ± 7.9	198.3 ± 10.8
High-density lipoprotein cholesterol, mg/dL	43.0 ± 2.2	41.0 ± 2.1	43.6 ± 2.3	42.2 ± 2.1
Triglyceride, mg/dL	144.1 ± 10.7	137.1 ± 12.7	140.1 ± 10.7	132.0 ± 10.9
Fasting plasma glucose mg/dL	98.4 ± 1.3	94.6 ± 1.3	97.6 ± 1.4	90.3 ± 1.1
Hemoglobin A1c, %	5.7 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.27 ± 0.1
Total sleep time, minutes	368.1 ± 20.3*	348.1 ± 20.3*	440.3 ± 20.9	414.3 ± 20.9
Apnea—hypopnea index, events/h	51.6 ± 3.0*	50.1 ± 3.2*	3.8 ± 0.3	3.6 ± 0.4
Lowest oxygen saturation, %	67.2 ± 2.1*	68.9 ± 3.0*	95.8 ± 0.5	94.1 ± 0.5
Oxyhemoglobin desaturation index	2.45 ± 0.32*	2.41 ± 0.36*	0.02 ± 0.01	0.02 ± 0.01
Arousal index, per hour	42.4 ± 3.1*	40.2 ± 2.2*	8.3 ± 3.1	8.3 ± 3.1
Circulating levels of soluble CD40 ligand, ng/mL	6.8 ± 3.7*	7.1 ± 4.1*	3.8 ± 1.2	4.2 ± 1.1
Circulating levels of high-sensitivity C-reactive protein, ng/mL	3.4 ± 1.1*	4.1 ± 2.1	1.6 ± 0.6	2.7 ± 1.4

*$P<.001$ versus control group.

not significantly different from those in elderly controls. hsCRP levels in middle-aged patients with OSAS were slightly but significantly higher than those in middle-aged controls. Because the insidious inflammatory responses were significantly different between middle-aged and elderly patients, there were more confounding factors affecting hsCRP levels in elderly subjects. Although systemic inflammation and cardiovascular events were frequently found in OSAS patients, the polysomnographic parameters and symptomatic variables did not clearly assess these changes. sCD40L, but not hsCRP, levels were associated with OSAS severity in elderly patients. Thus, CD 40 L level may be a better, more-sensitive marker for the practical assessment of inflammatory status and atherosclerotic progression in OSAS than hsCRP in elderly people.

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ACKNOWLEDGMENTS

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the author and has determined that none of the authors have any financial or any other kind of personal conflicts with this paper.

Financial Disclosure: Dr. Teramoto was supported by a research grant from Mitsui Life Social Welfare Foundation Japan fund and by research grant from Mitsukoshi Health and Welfare Foundation in Japan.

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Sponsor's Role: No sponsor in this study.

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STATEMENT OF INTEREST
None declared.

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DOI: 10.1183/09031936.00154307

Variation in the tumour necrosis factor-α gene is not associated with susceptibility to Asian COPD

To the Editors:

In a recent issue of the *European Respiratory Journal*, CHAPPELL *et al.* [1] clearly demonstrated that the lack of association with any of the tumour necrosis factor (TNF)-α single nucleotide polymorphisms or haplotypes makes it highly unlikely that polymorphisms in this gene play a major role in susceptibility to chronic obstructive pulmonary disease (COPD).

Their sample sizes were sufficient to elucidate association of TNF-α gene variations with susceptibility to COPD. However, they neglected the effect of ethnicity differences in genetic susceptibility to COPD. The frequency of the TNF-α-308*2 allele in Caucasian control populations (10–17%) is higher than that in Asians (0–8%) [1–10].

Genetic susceptibility to COPD is dependent upon the action of several gene polymorphisms, sex, age and ethnicity [2]. The TNF-α gene is known to have a polymorphic site at position -308. The TNF-α-308*2 allele, which is associated with a higher level of TNF-α production, has been associated with chronic bronchitis, a characteristic part of COPD, in a Taiwanese population [3]. However, the association of a polymorphism of TNF-α with susceptibility to COPD or to tobacco-related airway inflammation has not yet been confirmed in Asians. It was investigated whether the TNF-α-308*2 allele was associated with COPD in a Japanese population using a PCR-based genotyping assay [4]. The TNF-α-308*2 allele was found in one (1.9%) out of 53 patients with COPD and in one (1.5%) out of 65 smoker control subjects without COPD [4]. The frequency of the major allele, *i.e.* TNF-α-308*1, in the smoker control subjects (0.99) was consistent with data reported previously for other Japanese populations, suggesting that the present samples are representative of TNF-α gene polymorphism in the Japanese population [5]. However, there were no differences between COPD patients and smoker control subjects regarding the allele and genotype frequency of TNF-α. Since chronic bronchitis is not exactly the same, in terms of definition and tobacco sensitivity, as pulmonary emphysema, which is a major feature of COPD, it is possible that the TNF-α polymorphism is associated with infection-related bronchitis rather than tobacco-smoke-related alveolar wall destruction. However, most of the TNF polymorphism studies investigating COPD susceptibility revealed negative results for various Asian populations (table 1) [6–9]. Only one group of authors have insisted that the TNF-α-308*2 may be partly associated with the extent of emphysematous changes in patients with COPD [9].

TABLE 1 Association of tumour necrosis factor (TNF)-α gene polymorphism with chronic obstructive pulmonary disease (COPD) in Asian patients in various studies

First author [Ref.]	Race	Subjects n			Gene position	TNF-α A allele frequency %			Association with COPD
		COPD	SC	PC		COPD	SC	PC	
HUANG [3]	Taiwanese	42	42	99	TNF-α-308*2	19	2.4**	5.1**	Significant
ISHII [4]	Japanese	53	65		TNF-α-308*2	1.9	1.5		NS
SAKAO [9]	Japanese	106	110	129	TNF-α-308*2	16.5	8.2**	7.8**	Significant
CHIERAKUL [6]	Thai	57	67		TNF-α-308*2	7.9	4.7#		NS
JIANG [7]	Han Chinese (Beijing)	57	208		TNF-α-308	5.8	3.1¶		NS
HEGAB [8]	Japanese	88	61		TNF-α-308	2	0		NS

A allele: adenine allele (allele 2); SC: smoker control; PC: population control; TNF-α-308*2: position -308 on TNF-α allele 2; NS: nonsignificant. **: $p<0.001$; #: $p=0.14$; ¶: $p=0.131$.

Among Caucasian data, most of the TNF-α polymorphism studies investigating COPD susceptibility have revealed negative results. However, several authors have insisted that homozygosity of this allele predisposes the patient to more severe airflow obstruction and a worse prognosis in a small number of COPD cases [10].

Thus it is necessary to study polymorphisms within the tumour necrosis factor-α gene in a large collection of well-characterised Asian chronic obstructive pulmonary disease patients and control subjects, as in the European Union collaborative project [1]. However, these collective data strongly suggest that tumour necrosis factor-α gene polymorphism does not play a major role as a genetic risk factor for chronic obstructive pulmonary disease in either Caucasian or Asian individuals. However, the tumour necrosis factor-α gene polymorphism may be associated with functional impairment or prognosis in chronic obstructive pulmonary disease.

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STATEMENT OF INTEREST

None declared.

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DOI: 10.1183/09031936.00130607

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

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DOI: 10.1111/j.1743-6109.2008.00821.x

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 (IIEF-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.

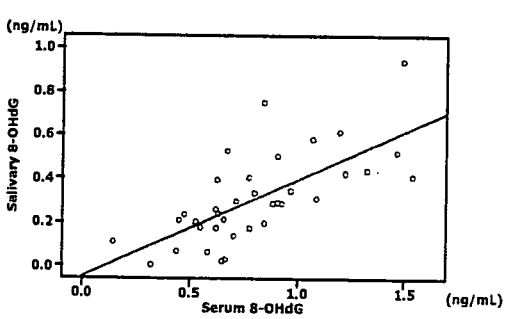

Figure 1 Correlation between salivary and serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels. There was a relatively good correlation between salivary and serum 8-OHdG levels (Pearson's correlation). N = 36, $r = 0.70$, $P < 0.001$, $y = 0.45x - 0.05$.

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.

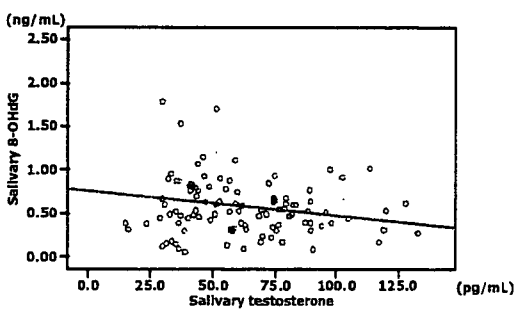

Figure 2 Association between salivary testosterone and salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels. There was a weak inverse association between salivary testosterone and 8-OHdG levels (Pearson's correlation). N = 128, $r = -0.21$, $P = 0.034$, $y = -0.003x + 0.766$.

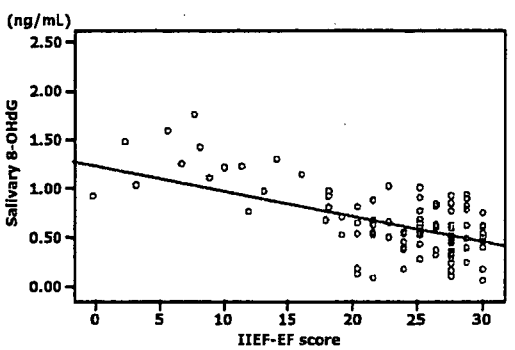

Figure 3 Association between the International Index of Erectile Function-erectile function domain (IIEF-EF) score and salivary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels. IIEF-EF scores significantly increased as salivary 8-OHdG levels decreased (Spearman's correlation). N = 128, $r = -0.33$, $P =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

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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Conflict of Interest: None declared.

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