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Association of cortisol and dehydroepiandrosterone sulphate levels in serum with periodontal status in older Japanese adults

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Abstract

Background and Aim: The associations between periodontitis and stress-related steroid hormone levels released by the hypothalamic–pituitary–adrenal axis are poorly understood. In this study, we examined the association between levels of the stress-related steroid hormones cortisol and dehydroepiandrosterone-sulphate (DHEAS) and periodontitis in elderly subjects.

Methods: A total of 467 subjects participated in this study. Serum cortisol and DHEAS levels were determined, and a medical questionnaire regarding medical conditions and lifestyle was administered. In addition, clinical examinations including probing depth (PD), bleeding on probing (BOP), and clinical attachment loss (CAL) were conducted.

Results: The subjects were divided into tertiles on the basis of periodontitis severity. When the analysis was stratified by smoking status, we found that cortisol levels were significantly higher in those with severe CAL among subjects who had never smoked. Furthermore, multiple regression analysis showed that a higher level of cortisol was significantly associated with greater numbers of sites with severe CAL only in those who had never smoked, while a somewhat weaker association was also observed regarding cortisol/DHEAS ratio. In contrast, the level of DHEAS in serum was not associated with periodontitis.

Conclusion: There were significant associations between serum cortisol level, including cortisol/DHEAS ratio, and periodontitis severity in elderly subjects who had never smoked.

Key words: cortisol; dehydroepiandrosterone sulphate; elderly; periodontitis; serum

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Conflict of interest and source of funding statement

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Stress can affect many aspects of physiology, while emotional status and the means of coping with stress can also influence health and disease. The stress system consists of brain elements, of which the main components are the corticotrophin-releasing hormone (CRH) and locus ceruleus (LC)-norepinephrine (NE)/autonomic systems, as well as their peripheral effectors, the hypothalamic–pituitary–adrenal (HPA) axis and autonomic system.

Many investigators have proposed an association between periodontitis, a chronic inflammatory disease, and psychosocial stress, with a systematic review recently presented (Peruzzo et al. 2007), in which the majority of

studies reviewed found a positive relationship between stress/psychological factors and periodontal disease. The biological mechanisms for such an association can be explained, at least in part, by host immune response, which makes the individual more susceptible to the development of unhealthy conditions and can affect periodontal health. Several studies have shown that psychological stress can regulate cellular immune responses via the HPA axis through the release of glucocorticoids, induction of the sympathetic nervous system, and release of neuropeptides from sensory nerve fibers (Marcenes & Sheiham 1992, Freeman & Goss 1993, Breivik et al. 1996, Monteiro da Silva et al. 1996).

In general, dysregulation of the stress system is expressed as either hyper- or hypoactivation of the HPA axis and LC/NE system. A group of disorders, including severe chronic disease, melancholic depression, and panic anxiety, may be associated with an increased and prolonged activation of the HPA axis, while another group, which includes atypical seasonal depression and chronic fatigue syndrome, is characterized by hypoactivation of the stress system (Chrousos & Gold 1992).

Cortisol is an end-product of the HPA axis. When stimulated, the hypothalamus secretes CRH and, in response, the pituitary gland secretes adrenocorticotropic hormone (ACTH), which in turn stimulates the secretion of cortisol from the cortex of the adrenal gland. Cortisol circulates in the blood in both free and bound forms, and is predominantly bound to corticosteroid-binding globulin in plasma, while the remainder is free (Levine et al. 2007). On the other hand, another ACTH-dependent hormone, dehydroepiandrosterone (DHEA), also known as DHEA-sulphate (DHEAS), is also affected by dysregulation of the stress system (Heuser et al. 1998, Michael et al. 2000, Hsiao 2006). Also, a recent study reported that the cortisol/DHEA(S) molar ratio was a useful marker of anxiety and depressive illness (Ritsner et al. 2007). However, little is known regarding periodontal status and HPA axis response, and to the best of our knowledge, there are only three studies, including our own, of cortisol levels (Mengel et al. 2002, Hilgert et al. 2006, Ishisaka et al. 2007), of which the study by Mengel et al. (2002) was the only one to use serum samples. As for the levels of DHEA in saliva, ours is the only one to have been presented thus far (Ishisaka et al. 2007).

Smoking is a major risk factor for periodontal disease (Tomar & Asma 2000), while it was found to be associated with an increase in cortisol levels, and considered likely to be a significant mediator in the relationship between stress-related disorders and the HPA axis (Olff et al. 2006). Confounding and effect modification is of increasing importance as periodontal researchers address the putative associations between periodontal disease and systematic diseases, and is especially pertinent when dealing with smoking, because it is a major risk factor for both periodontal disease and a number of systematic diseases (Hyman 2006).

In the present cross-sectional study, we analysed the association between periodontitis and the HPA axis using cortisol and DHEAS in serum as markers, with the results stratified by smoking status. Furthermore, we investigated whether periodontitis severity was linked to changes in the levels of steroids in the same subjects.

Material and Methods

Study population

A total of 624 individuals (293 males, 331 females; age range 60 to 81 years) residing in Fukuoka Prefecture, Japan, were invited to participate in the study. All were physically and mentally healthy, and independent in daily activities, with none hospitalized at the time of the study. Exclusion criteria were as follows: (1) individuals who used antibiotics within the last 6 months, had symptoms of acute illness (e.g. fever and sore throat), or any apparent oral infection; (2) individuals with missing questionnaires or serum samples; (3) individuals over 70 years old (in order to focus on a younger cohort); and (4) individuals with fewer than 10 natural teeth. As a result, we analysed 467 subjects (217 males, 250 females), aged 60–69 years old (mean: 62.6 ± 2.89 years).

Before beginning the examinations, each subject was asked to respond to a survey consisting of questions related to general medical condition, medication usage, lifestyle, oral health behaviour, oral hygiene habits, and smoking status (current, past, or never). We classified the subjects as current-smokers, past-smokers, and never-smokers based on their interview responses. A method that used face-scale scores was used to evaluate self-rated health status (Lorish & Maisiak 1986). From the scores of the answers, the subjects were divided into three groups based on overall health (moderate, good, and very good). The study was approved by the Ethics Committee of Kyushu Dental College (no. 05022250) and all subjects provided written informed consent before participation.

Laboratory testing

The subjects were instructed to abstain from unusual physical activity before blood collection. Blood samples were taken between 9:00 am and 11:00 am after 20 min. of rest, after they had refrained from oral intake and smoking for at least 2 h before collection, then centrifuged to obtain serum, which was stored at -80°C until the day of the assay. Total cortisol and DHEAS levels in serum were measured using a radioimmunoassay method by a commercial laboratory (SRL Inc., Tokyo, Japan). Cortisol level in serum was detected and considered to be normal in a range from 110.36 to 504.90 nmol/l, while DHEAS (60 years old over) was considered to be normal in a range from 651.36 to 6622.16 nmol/l for males, 325.68 to 3609.62 nmol/l for females.

Clinical examination

Periodontal status was evaluated using probing depth (PD), bleeding on probing (BOP), and clinical attachment loss (CAL). Periodontal examinations were conducted at 2 sites (mesio-buccal and mid-buccal) of each tooth using a standard periodontal probe (Hu-Friedy, Chicago, USA), which was inserted into the periodontal pocket parallel to the long axis of the tooth for all teeth fully erupted in the mouth, according to the method described in the Third National Health and Nutrition Examination Survey (NHANES III) (Albandar et al. 1999), with some modifications. All periodontal examinations were performed by two dentists. To confirm inter-examiner reliability, duplicate examinations were conducted with outpatients visiting Kyushu Dental College Hospital. The kappa values between the examiners for assessment of PD and CAL were 0.95 and 0.72, respectively. Severe periodontitis was defined as PD

of at least 4 mm or more or CAL of at least 5 mm. Furthermore, we divided the subjects into tertiles according to the proportion of sites with PD $\geqslant$ 4 mm or CAL $\geqslant$ 5 mm, in order to analyse the association between the extent of periodontitis and serum levels of the stress-related steroid hormones, which were: none, no sites (first tertile); low, second tertile with PD $\geqslant$ 4 mm or CAL $\geqslant$ 5 mm; and high, third tertile with PD $\geqslant$ 4 mm or CAL $\geqslant$ 5 mm.

Statistical analyses

The median, and 25th and 75th percentiles for clinical parameters and serum values were determined for each group. Kruskal–Wallis tests were used to examine continuous variables, as a normal distribution was not present according to the results of a Kolomogorov–Smirnov test. If necessary, a Scheffe test and Steel–Dwass test were used for multiple comparisons following the Kruskal–Wallis test. Categorical variables were compared using a chi-square test. Pearson correlation coefficients were used to compare the hormone levels to the other parameters. In addition, we performed multiple regression analyses separately for PD and CAL to establish their respective associations with the levels of cortisol and DHEAS, and cortisol/DHEAS (*100) molar ratio, with adjustments for potential confounders, which showed an association ($p < 0.20$) by univariate analysis, including those with clinical epidemiological relevance, including age, gender, diabetes, oral hygiene habits (frequency of tooth brushing per day), and mean BOP (final model). Gender, diabetic status, oral hygiene habits, and BOP (two categories) were converted to dummy variables. All analyses were stratified by smoking status. All statistical analyses were performed using SPSS 11.0 for Windows (SPSS Japan, Tokyo, Japan), with the level of statistical significance was set at 0.05 for all of the analyses.

Results

The mean cortisol and DHEAS levels in serum samples from all of the subjects were 419.62 ± 182.15 (SD) nmol/l (range 27.59 to 1407.09 nmol/l) and 3583.57 ± 2104.96 (SD) nmol/l (range 81.42 to 13,162.90 nmol/l), respectively. There were significant differences between males and females regarding those levels. Furthermore, there was a significant correlation between age and DHEAS concentration ($r = -0.098$, $p = 0.034$), while there was none between age and cortisol ($r = 0.035$, $p = 0.45$).

The characteristics of the subjects divided by tertiles are shown in Table 1. For PD and CAL, there were significant differences among age, number of teeth, maximum PD, maximum CAL, mean BOP, gender, smoking status, and diabetes. Conversely, there were no significant differences with regard to the use of an interdental brush, dental visits in the past 12 months, and self-rated health status (based on face-scale scores) for those parameters for PD, whereas there were significant differences among the values for frequency of tooth brushing per day.

Next, we compared the levels of the stress hormones cortisol and DHEAS in serum, and determined the cortisol/DHEAS molar ratio by PD and CAL levels tertiles, then stratified by smoking status, as shown in Tables 2 and 3, respectively. As for the never-smokers, significant differences were found only among the CAL tertiles with regard to the level of cortisol, whereas no significant associations were found with regard to the level of DHEAS or cortisol/DHEAS molar ratio, regardless of the PD and CAL values. With regard to the levels of cortisol and DHEAS, there were significant associations among the three stratifications by smoking (data not shown), which indicated that smoking causes an increase in cortisol and DHEAS. From these findings, it was considered reasonable that smoking be treated as an effect modifier in the following analyses.

To determine whether levels of cortisol, DHEAS, and cortisol/DHEAS molar ratio were associated with the severity and extent of periodontitis, we performed multiple regression analyses stratified by smoking status, with adjustment for various potential confounding variables. Sequential models for the associations between these stress-related hormone levels and the groups assessed by PD and CAL, which were stratified by smoking status, are shown in Tables 4 and 5, respectively. As for PD, there were no significant associations between the level of cortisol and DHEAS, including the cortisol/DHEAS molar ratio, with any of the models, regardless of smoking status. In contrast, for CAL, the level of cortisol was associated significantly with both the second and third tertiles with both models in never-smokers, even after adjustment for potential confounding factors, and a stronger association was seen with the third tertile. Significant associations were also found between the cortisol/DHEAS molar ratio and second tertile in never-smokers with the final models. There were no significant associations between periodontal status and DHEAS level with any of the models.

Discussion

In the present cross-sectional-study, we investigated HPA response and periodontal status in relatively healthy elderly subjects, and found that serum levels of cortisol were associated with periodontitis severity, as shown by CAL, in subjects who had never smoked, whereas a somewhat weaker association with cortisol/DHEAS molar ratio was observed, while the level of DHEAS in serum was not associated with periodontal status.

The association between periodontitis and stress-related hormones has been largely overlooked in dental research, with only three human studies of the associations between cortisol and periodontal disease reported. Two of those were presented in a recent systematic review (Peruzzo et al. 2007) and the other was our own survey, which found close relationships between periodontal status and salivary levels of cortisol and DHEA in elderly subjects (Ishisaka et al. 2007). In addition, another report regarding the positive association between salivary cortisol and periodontitis was presented (Hilgert et al. 2006), in which hypercortisolemia was found to be independently associated with the severity of periodontitis, as defined by CAL (mean CAL $\geqslant$ 4 mm vs. $<$ 4 mm), and the extent of periodontitis, as defined by PD ($\geqslant$ 26% vs. $<$ 26% of the sites with PD $\geqslant$ 4 mm) or CAL (at least 30% of the sites having at least 5 mm of CAL vs. less than 30%). In a separate study (Mengel et al. 2002), no correlations were found among serum cortisol, a pessimistic attitude to life (assessed by questionnaire), and levels of immunological mediators (IL-1β, IL-6). Those latter findings may have been due to the small sample size, as the test group of subjects with periodontitis and control group composed of periodontal healthy individuals recruited from a

Table 1. Subject characteristics according to PD and CAL levels

Characteristics	Sites with PD ⩾ 4 mm*				Sites with CAL ⩾ 5 mm*			
	none	low	high	*p* value	none	low	high	*p* value
Number	159	162	146		188	130	149	
Age (years)								
Median	60.0	63.0	64.5	<0.001	60.0	63.0	65.0	<0.001
(25th, 75th percentile)	(60.0, 63.0)	(60.0, 65.0)	(60.0, 65.0)		(60.0, 65.0)	(60.0, 65.0)	(60.0, 65.0)	
Number of teeth								
Median	27.0	26.0	24.5	0.001	27.0	27.0	23.0	<0.001
(25th, 75th percentile)	(23.0, 28.0)	(23.0, 28,0)	(19.0, 27.0)		(24.0, 28.0)	(24.0, 28.0)	(19.0, 26.0)	
Maximum PD (mm)								
Median	3.0	4.0	5.0	<0.001	3.0	4.0	5.0	<0.001
(25th, 75th percentile)	(3.0, 3.0)	(4.0, 5.0)	(4.0, 6.0)		(3.0, 4.0)	(3.0, 5.0)	(4.0, 6.0)	
Maximum CAL (mm)								
Median	4.0	5.0	6.0	<0.001	4.0	5.0	6.0	<0.001
(25th, 75th percentile)	(3.0, 5.0)	(4.0, 6.0)	(5.0, 7.0)		(3.0, 4.0)	(5.0, 6.0)	(6.0, 8.0)	
Percent of sites with BOP								
Median	2	4	5	<0.001	4	4	5	0.001
(25th, 75th percentile)	(0, 6)	(0, 11)	(2, 13)		(0, 8)	(0, 9)	(2, 15)	
Sex								
Male	65 (41)	68 (42)	84 (58)	0.01	65 (35)	61 (47)	91 (61)	<0.001
Female	94 (59)	94 (58)	62 (42)		123 (65)	69 (53)	58 (39)	
Smoking status								
Current	16 (10)	13 (8)	21 (14)	0.10	17 (9)	11 (8)	22 (15)	0.001
Past	34 (22)	42 (26)	45 (31)		34 (18)	36 (28)	51 (34)	
Never	107 (68)	107 (66)	80 (55)		136 (73)	83 (64)	75 (51)	
Diabetes								
Yes	3 (2)	9 (6)	12 (8)	0.04	5 (3)	4 (3)	15 (10)	0.004
No	156 (98)	153 (94)	134 (92)		183 (97)	126 (97)	134 (90)	
Use of interdental brush								
Yes	69 (43)	79 (49)	63 (43)	0.26	84 (45)	64 (49)	67 (45)	0.69
No	90 (57)	83 (51)	83 (57)		104 (55)	66 (51)	82 (55)	
Dental visit in past 12 months								
Yes	85 (53)	92 (57)	64 (44)	0.06	93 (50)	67 (52)	81 (54)	0.70
No	74 (47)	69 (43)	82 (56)		94 (50)	63 (48)	68 (46)	
Frequency of tooth brushing (per day)								
⩽1	46 (29)	37 (23)	44 (30)	0.04	53 (28)	31 (24)	43 (29)	0.55
2	87 (55)	76 (47)	73 (50)		97 (52)	71 (55)	68 (46)	
⩾3	26 (16)	49 (30)	29 (20)		38 (20)	28 (22)	38 (26)	
Self-rated health status								
Very good	120 (75)	115 (71)	100 (68)	0.47	135 (72)	97 (75)	103 (70)	0.09
Good	33 (21)	38 (24)	42 (29)		41 (22)	29 (22)	43 (29)	
Moderate	6 (4)	8 (5)	4 (3)		12 (6)	4 (3)	2 (1)	

*Defined by the percent of sites (tertiles) with PD ⩾ 4 mm or CAL ⩾ 5 mm.
Categorical variables indicate the number of subjects (%).
Differences between groups were tested using χ^2 test for categorical variables and a Kruskal–Wallis test for continuous variables.
PD, probing depth (mm); CAL, clinical attachment loss (mm); BOP, bleeding on probing.

recall program consisted of only 40 subjects each. On the other hand, an animal experiment showed an association between higher serum corticosterone levels and ligature-induced periodontitis in depression model rats (Breivik et al. 2006).

The present results indicate that serum levels of cortisol are associated with periodontitis severity shown by CAL in individuals who have never smoked, though no significant associations between cortisol and periodontitis severity shown by PD were found. These findings may be explained as follows. As noted in a previous study (Chrousos & Gold 1992), severe chronic diseases are disorders related to hyperfunction of the HPA axis response. Because CAL level can be regarded as a result of an inflammatory burden from the past into the present, in contrast to PD level which reflects the current pathophysiological status of periodontitis, the findings from our study may be attributed to dysregulation of the stress system, in which the HPA axis is chronically activated in patients with severe periodontitis. Several animal studies have also revealed that HPA axis overresponsiveness is associated with increased susceptibility to periodontitis (Breivik et al. 2000). Thus, we propose that severe periodontitis be considered as one of the severe chronic diseases associated with increased stress system activity, which was previously reported by Chrousos and Gold (1992).

The cellular and molecular bases for the interactions between stress hormones and periodontitis can be explained by activities of the HPA axis, such as promoting the release of CRH from the hypothalamus and glucocorticoids from the adrenal cortex (Genco et al. 1998). Glucocorticoids, including cortisol (the primary glucocorticoid), exert major suppressive effects through highly

Table 2. Median values for serum stress hormones in presence or absence of extensive periodontitis, separated by PD across smoking status

	Current				Past				Never			
	sites with PD ⩾ 4 mm*				sites with PD ⩾ 4 mm*				sites with PD ⩾ 4 mm*			
	none	low	high	*p* value†	none	low	high	*p* value†	none	low	high	*p* value†
Number	16	13	21		34	42	45		107	107	80	
Cortisol (nmol/l)												
Median (25th, 75th percentile)	393.16 (303.2, 525.6)	333.84 (198.6, 491.1)	441.4 (289.7, 586.3)	0.29	441.44 (320.7, 517.3)	460.75 (357.3, 568.4)	460.8 (314.5, 589.0)	0.51	364.19 (273.1, 485.6)	342.12 (270.4, 458.0)	389.02 (325.6, 524.2)	0.11
DHEAS (nmol/l)												
Median (25th, 75th percentile)	6106.50 (3446.8, 7694.2)	3148.2 (2534.9, 5034.5)	4858.06 (2917.6, 7368.5)	0.10	4559.52 (3304.3, 5862.2)	4410.25 (3080.4, 6737.5)	4125.28 (2095.2, 5916.5)	0.48	2667.86 (1769.5, 3473.9)	2768.28 (1948.7, 3908.2)	2632.6 (1842.1, 3867.5)	0.65
Cortisol/DHEAS ratio(∗100)												
Median (25th, 75th percentile)	7.67 (5.1, 8.9)	8.11 (6.7, 15.9)	9.03 (5.3, 16.5)	0.55	9.55 (6.4, 16.7)	10.28 (7.1, 17.0)	11.89 (7.1, 19.3)	0.48	14.57 (9.7, 20.2)	12.99 (8.6, 18.1)	15.84 (10.7, 24.6)	0.19

*Defined by the percent with sites (tertiles) with PD ⩾ 4 mm .
†Kruskal–Wallis test.
DHEAS, dehydroepiandrosterone sulphate; PD, probing depth (mm).

Table 3. Median values for serum stress hormones in presence or absence of extensive periodontitis, separated by CAL across smoking status

	Current				Past				Never			
	sites with CAL ⩾ 5 mm*				sites with CAL ⩾ 5 mm*				sites with CAL ⩾ 5 mm*			
	none	low	high	*p* value†	none	low	high	*p* value†	none	low	high	*p* value†
Number	17	11	22		34	36	51		136	83	75	
Cortisol (nmol/l)												
Median (25th, 75th percentile)	419.37 (306.2, 492.5)	386.26 (289.7, 554.6)	413.9 (234.5, 550.4)	0.84	437.30 (323.5, 573.2)	437.16 (330.4, 549.7)	460.75 (361.4, 571.1)	0.77	332.46 (249.0, 460.1)	389.02 (320.0, 535.2)	391.78 (311.8, 524.2)	0.001
DHEAS (nmol/l)												
Median (25th, 75th percentile)	5020.90 (2575.6, 6703.6)	5563.70 (2795.4, 6703.6)	4722.36 (2890.4, 7232.8)	0.90	4559.5 (3087.2, 6255.8)	4545.95 (3161.8, 5726.5)	4016.7 (2518.6, 6296.5)	0.87	2609.5 (1789.9, 3514.6)	2714.00 (1709.8, 3935.3)	2876.84 (1959.5, 3826.7)	0.55
Cortisol/DHEAS ratio(∗100)												
Median (25th, 75th percentile)	8.11 (6.1, 19.0)	8.27 (4.7, 13.7)	8.20 (5.4, 15.4)	0.74	10.03 (7.3, 16.0)	7.99 (6.1, 14.4)	12.38 (7.1, 19.6)	0.23	12.83 (8.3, 18.9)	15.25 (10.2, 22.7)	14.16 (11.0, 20.9)	0.10

*Defined by the percent with sites (tertiles) with CAL ⩾ 5 mm.
†Kruskal–Wallis test.
DHEAS, dehydroepiandrosterone sulphate; CAL, clinical attachment loss (mm).

specific mechanisms at multiple levels, for example, by inhibiting the cascade of immune response (Snyder & Unanue 1982) and production of cytokines (Williams & Yarwood 1990). A number of studies have shown the contribution of Th-1 and Th-2 cytokines in periodontitis. However, at present no distinct difference between Th-1 and Th-2 cytokine profiles can be distinguished in periodontitis lesions, though it is highly likely that the balance of Th-1 and Th-2 cytokines is an important issue in disease expression (Berglundh & Donati 2005). Those changes have major suppressive effects on immune and inflammatory responses, and give rise to increased susceptibility, leading to the establishment of periodontal infection, which in turn results in destructive periodontitis (Peruzzo et al. 2007).

DHEAS is a neuroactive steroid produced by the adrenal cortex in response to ACTH (Kroboth et al. 1999). Both of these hormones are apparently interchangeable, though 99% of circulating DHEA is in the sulphate form. DHEAS concentrations are known to decline with age, which was confirmed in the present results. Furthermore, levels of the sulphate form in serum are 300–500 times higher and have less diurnal variation than the non-sulphate form (Ebeling & Koivisto 1994, Leowattana 2004). A previous study reported that levels of both forms may more adequately reflect HPA dysregulation as compared with cortisol (Fabian et al. 2001), while another (Hechter et al.

Table 4. Multiple regression analysis of the effects of explanatory variables including PD on serum stress hormones, stratified by smoking status

	Current									Past							Never							
	dependent variables									dependent variables							dependent variables							
	cortisol			DHEAS			cortisol/ DHEAS ratio			cortisol			DHEAS			cortisol/ DHEAS ratio			cortisol			DHEAS			cortisol/DHEAS ratio		
	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p
Number of subjects					50							121									294					
Crude																										
Low (second tertile)	−0.20	−1.20	0.24	−0.34	−2.13	0.04	0.20	1.23	0.23	0.14	1.26	0.21	0.05	0.49	0.63	0.09	0.78	0.44	−0.03	−0.52	0.60	0.06	0.82	0.42	−0.03	−0.46	0.64
High (third tertile)	0.05	0.27	0.79	−0.12	−0.74	0.47	0.16	0.99	0.33	0.05	0.44	0.66	−0.10	−0.93	0.36	0.12	1.05	0.30	0.12	1.79	0.08	0.06	0.85	0.40	0.04	0.58	0.57
Final model																											
Low (second tertile)	−0.03	−0.17	0.86	−0.25	−1.56	0.13	0.18	1.03	0.31	0.11	0.93	0.35	0.03	0.22	0.82	0.08	0.69	0.49	−0.03	−0.43	0.67	0.09	1.39	0.17	−0.04	−0.59	0.55
High (third tertile)	0.14	0.83	0.41	−0.04	−0.28	0.78	0.16	0.90	0.37	−0.01	−0.04	0.97	−0.12	−1.08	0.28	0.10	0.81	0.42	0.10	1.39	0.17	0.04	0.64	0.53	0.03	0.48	0.63

Final model: Adjusted for age, gender, diabetes, frequency of tooth brushing (per day), and BOP.
Reference is the first tertile (i.e. no sites with PD $\geqslant$ 4 mm).
PD, probing depth; β, regression coefficient; DHEAS, dehydroepiandrosterone sulphate; BOP, bleeding on probing.

1997) postulated that DHEA(S) maintains cortisol homeostasis by serving as an anti-glucocorticoid in humans. Therefore, DHEA(S) might block the development of the diverse pathological processes potentiated by a prolonged increase in cortisol secretion. Several studies have suggested that the cortisol/DHEAS molar ratio may influence the interrelationship between these steroids and individuals with an elevated ratio may be at risk for psychiatric disorders (Michael et al. 2000, Ritsner et al. 2007). Although several studies of serum DHEAS and cortisol/DHEAS molar ratio have been published, no clear pattern has emerged, as increased, normal, and decreased levels of DHEAS and cortisol/DHEAS ratio have been found (Fabian et al. 2001, Harris et al. 2001, Shirayama et al. 2002, Young et al. 2002, Ritsner et al. 2007). In the present study, we found a significant association between cortisol/DHEAS molar ratio and periodontal status shown by CAL, though that was somewhat weaker than the association with cortisol level. Our results indicate that cortisol/DHEAS molar ratio should be considered as a candidate of risk marker for periodontitis, though it is limited to individuals who have never smoked.

The primary goal of our study was to examine the biologically plausible hypothesis that these hormones can be used as predictors of periodontitis, though the present results are limited because of the cross-sectional nature of the study. We performed a preliminary analysis using receiver operating characteristics (ROC) analysis to describe false-positive *versus* true-positive diagnosis of periodontitis (i.e. second and third tertiles) at various cut-off levels of cortisol, in subjects who never smoked. As a result, the area under the curve (AUC) was 0.628 (95% confidence interval, 0.564–0.692, $p < 0.001$). The optimal sensitivity and specificity of cortisol were found to be 0.60 and 0.62, respectively, when the cut-off value was 368.33 nmol/l, which indicated a corresponding level of the median (366.95 nmol/l) of cortisol in never-smokers. It may be possible to differentiate periodontitis affected patients from periodontal- healthy individuals by measuring cortisol levels in a selected population (e.g. never-smokers).

Smoking is a major risk factor for severe periodontal disease (Tomar & Asma 2000). In a recent study, the increase in risk was averaged over

Table 5. Multiple regression analysis of the effects of explanatory variables including CAL on serum stress hormones, stratified by smoking status

	Current								Past								Never							
	dependent variables								dependent variables								dependent variables							
	cortisol			DHEAS			cortisol/DHEAS ratio		cortisol			DHEAS			cortisol/ DHEAS ratio			cortisol			DHEAS			cortisol/ DHEAS ratio	
	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p
Number of subjects	50								121								294							
Crude																								
Low (second tertile)	0.05	0.30	0.77	0.12	0.70	0.49	0.02	0.12	0.90	−0.04	−0.32	0.75	0.07	0.60	0.55	0.04	0.34	0.74	0.16	2.66	0.01	0.08	1.24	0.22	0.12	1.97	0.050
High (third tertile)	−0.08	−0.50	0.62	0.09	0.55	0.59	−0.14	−0.88	0.39	0.02	0.17	0.87	−0.01	−0.10	0.92	0.09	0.81	0.42	0.22	3.57	<0.001	0.09	1.44	0.15	0.06	0.94	0.35
Final model																											
Low (second tertile)	0.13	0.77	0.45	0.21	1.41	0.17	−0.01	−0.03	0.97	0.01	−0.07	0.95	0.12	1.11	0.27	0.02	0.22	0.83	0.16	2.57	0.01	0.04	0.74	0.46	0.14	2.27	0.02
High (third tertile)	0.04	0.26	0.80	−0.00	−0.02	0.99	−0.02	−0.14	0.89	−0.02	−0.21	0.84	−0.03	−0.27	0.79	0.07	0.62	0.54	0.21	3.24	0.001	0.04	0.67	0.50	0.08	1.28	0.20

Final model: adjusted for age, gender, diabetes, frequency of tooth brushing (per day), and BOP.
Reference is the first tertile (i.e. no sites with CAL $\geqslant$ 5 mm).
CAL, clinical attachment loss; β, regression coefficient; DHEAS, dehydroepiandrosterone sulphate; BOP, bleeding on probing.

both smokers and non-smokers, thus treating smoking as a confounder also resulted in a greatly underestimated magnitude of association (Hyman & Reid 2004). Furthermore, another study found an association between advanced periodontal disease and self-reported coronary heart disease, however, after stratifying by smoking status, the association was reported to be limited to smokers (Hyman et al. 2002). In a very recent study, it was found that ignoring the effect modification would have resulted in a faulty analysis and incorrect inferences (Hyman 2006). Hujoel et al. (2002) also suggested that the periodontitis-systematic disease relationship should be studied using healthy subjects who have never smoked. In the present study, when smoking was treated as an effect modifier, our findings of an association between levels of cortisol, or cortisol/DHEAS molar ratio and periodontitis severity were largely limited to the never-smokers. These results suggest that the effects of smoking on changes in cortisol level and cortisol/DHEAS molar ratio as markers of periodontal status may be slight. Nevertheless, it is important to note that tobacco smoking and nicotine have pronounced effects on endocrine function as well, and smoking is known to increase cortisol levels (Steptoe & Ussher 2006). We found that the level of cortisol was increased in smokers as compared with subjects who had never smoked. Thus, because a strong interaction between smoking and cortisol level was found, cortisol does not appear to be an appropriate marker for periodontitis in individuals with a past history of habit of smoking.

In general, a periodontal probe is used for periodontitis screening during community-based oral health examinations, however, several problems, including cost, prevention of infection, and manpower needs, have been pointed out. Serum stress-related hormones could be utilized as part of the diagnostic criteria when screening for the presence of periodontitis and a special device that utilizes self-sampled fingertip plasma (50 µl) has been recently developed (Leisure Inc., Japan) (Iwasawa 2007). Assessment of biomarkers in a small amount of plasma collected with such a device may be a simple and useful alternative to the conventional probing method.

A limitation of the present study is that our subjects were generally in good

health, thus our findings may indicate that the association exists primarily in healthy elderly subjects, and that a cohort of these elderly individuals is not ideally suited to address stress-related effects in the pathology of periodontitis. Additional investigations are necessary to validate and extend the findings using subjects of other ages or with outpatients. Further, whether cortisol and/or the cortisol/DHEAS molar ratio are useful as predictors for periodontitis is unknown. The causes and effects of these biomarkers remain unclarified by the present results, as assessment of CRH and ACTH in the HPA axis system were not performed. A longitudinal study would be necessary to determine the relationships of stress-related hormones in serum to the progression of periodontitis.

In summary, we found significant associations between serum cortisol levels, including cortisol/DHEAS molar ratio, and periodontitis severity in healthy elderly subjects who had never smoked. Our results suggest that levels of cortisol and cortisol/DHEAS molar ratio are useful candidate biomarkers for evaluating at least a part of the etiopathogenesis of periodontitis. Investigations of other biomarkers may open new avenues for development of a screening test for periodontitis and monitoring the response to treatment in the near future.

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References

Albandar, J. M., Brunelle, J. A. & Kingman, A. (1999) Destructive periodontal disease in adults 30 years of age and older in the United States, 1988–1994. *Journal of Periodontology* **70**, 13–29.

Berglundh, T. & Donati, M. (2005) Aspects of adaptive host response in periodontitis. *Journal of Clinical Periodontology* **32** (Suppl 6), 87–107.

Breivik, T., Gunderse, Y., Myhrer, T., Fonnum, F., Osumundsen, H., Murison, R., Gjermo, P., von Hösten, S. & Opstad, PK. (2006) Enhanced susceptibility to periodontitis in an animal model of depression: reversed by chronic treatment with the anti-depressant tianptine. *Journal of Clinical Periodontology* **33**, 469–477.

Breivik, T., Opstad, P. K., Gjermo, P. & Thrane, P. S. (2000) Effects of hypothalamic-pituitary-adrenal axis reactivity on periodontal tissue destraction in rats. *European Journal of Oral Sciences* **108**, 115–122.

Breivik, T., Thrane, O., Murison, R. & Gjermo, P. (1996) Emotional stress effects on immunity, gingivitis and periodontits. *European Journal of Oral Sciences* **104**, 327–334.

Chrousos, G. P. & Gold, P. W. (1992) The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *The Journal of the American Medical Association* **267**, 1244–1252.

Ebeling, P. & Koivisto, V. A. (1994) Physiological importance of dehydroepiandrosterone. *Lancet* **343**, 1479–1481.

Fabian, T. J., Dew, M. A., Pollock, B. G., Reynolds, C. F. III, Mulsant, B. H., Butters, M. A., Zmuda, M. D., Linares, A. M., Trottini, M. & Kroboth, P. D. (2001) Endogenous concentrations of DHEA and DHEA-S decrease with remission of depression in older adults. *Biological Psychiatry* **50**, 767–774.

Freeman, R. & Goss, S. (1993) Stress measures as predictors of periodontal disease – a preliminary communication. *Community Dentistry and Oral Epidemiology* **21**, 176–177.

Genco, R. J., Ho, A. W., Kopman, J., Grossi, S. G., Dunford, R. G. & Tedesco, L. A. (1998) Models to evaluate the role of stress in periodontal disease. *Annals of Periodontology* **3**, 288–302.

Harris, D. S., Wolkowitz, O. M. & Reus, V.I (2001) Movement disorder, memory, psychiatric symptoms and serum DHEA levels in schizophrenic and schizoaffective patients. *The World Journal of Biological Psychiatry* **2**, 99–102.

Hechter, O., Grossman, A. & Chatterton, R. T. Jr. (1997) Relationship of dehydroepiandrosterone and cortisol in disease. *Medical Hypotheses* **49**, 85–91.

Heuser, I., Deuschle, M., Luppa, P., Schweiger, U., Standhardt, H. & Weber, B. (1998) Increased diurnal plasma concentrations of dehydroepiandrosterone in depressed patients. *The Journal of Clinical Endocrinology and Metabolism* **83**, 3130–3133.

Hilgert, J. B., Hugo, F. N., Bandeira, D. R. & Bozzetti, M. C. (2006) Stress, cortisol, and periodontitis in a population aged 50 years and over. *Journal of Dental Research* **85**, 324–328.

Hsiao, C. C. (2006) Positive correlation between anxiety severity and plasma levels of dehydroepiandrosterone sulfate in medication-free patients experiencing a major episode of depression. *Psychiatry and Clinical Neurosciences* **60**, 746–750.

Hujoel, P. P., Drangsholt, M., Spiekerman, C. & DeRouen, T. A. (2002) Periodontitis-systematic disease associations in the presence of smoking – Causal or coincidental? *Periodontology 2000* **30**, 51–60.

Hyman, J. (2006) The importance of assessing confounding and effect modification in research involving periodontal disease and systematic diseases. *Journal of Periodontology* **33**, 102–103.

Hyman, J. J. & Reid, B. C. (2004) Cigarette smoking, periodontal disease: and chronic obstructive pulmonary disease. *Journal of Periodontology* **75**, 9–15.

Hyman, J. J., Winn, D. M. & Reid, B. C. (2002) The role of cigarette smoking in the assiciation between periodontal disease and coronaryheart disease. *Journal of Periodontology* **73**, 988–994.

Ishisaka, A., Ansai, T., Soh, I., Inenaga, K., Yoshida, A., Shigeyama, C., Awano, S., Hamasaki, T., Sonoki, K., Takata, Y. & Takehara, T. (2007) Association of salivary levels of cortisol and dehydroepiandrosterone with periodontitis in older Japanese adults. *Journal of Periodontology* **78**, 1767–1773.

Iwasawa, H. (2007) Mail-medicine test for healthy check and the quality. *Kiki Shiyaku* **30**, 13–20 (in Japanese).

Kroboth, P. D., Salek, F. S., Pittenger, A. L., Fabian, T. J. & Frye, R. F. (1999) DHEA and DHEA-S: a review. *Journal of Clinical Pharmacology* **39**, 327–348.

Leowattana, W. (2004) DHEAS as a new diagnostic tool. *Clinica Chimica Acta: International Journal of Clinical Chemistry* **341**, 1–15.

Levine, A., Zagoory-Sharon, O., Feldman, R., Lewis, J. G. & Weller, A. (2007) Measuring cortisol in human psychobiological studies. *Physiology and Behavior* **90**, 43–53.

Lorish, C. D. & Maisiak, R. (1986) The face scale: a brief, nonverbal method for assessing patient mood. *Arthritis and Rheumatism* **29**, 906–909.

Marcenes, W. S. & Sheiham, A. (1992) The relationship between work stress and oral health status. *Social Science and Medicine* **35**, 1511–1520.

Mengel, R., Bacher, M. & Flores-De-Jacoby, L. (2002) Interactions between stress, interleukin-1beta, interleukin-6 and cortisol in periodontally diseased patients. *Journal of Clinical Periodontology* **29**, 1012–1022.

Michael, A., Jenaway, A., Paykel, E. S. & Herbert, J. (2000) Altered salivary dehydroepiandrosterone levels in major depression in adults. *Biological Psychiatry* **48**, 989–995.

Monteiro da Silva, A. M., Oakley, D. A., Newman, H. N., Nohl, F. S. & Lloyd, H. M. (1996) Psychosocial factors and adult onset rapidly progressive periodontitis. *Journal of Clinical Periodontology* **23**, 789–794.

Olff, M., Meewisse, M. L., Kleber, R. J., van der Velden, P. G., Drogendijk, A. N., van Amsterdam, J. G., Opperhuizen, A. & Gersons, B. P. (2006) Tobacco usage interacts with postdisaster psychopathology on circadian salivary cortisol. *International Journal of Psychophysiology* **59**, 251–258.

Peruzzo, D. C., Benatti, B. B., Ambrosano, G. M. B., Nogueira-Filho, G. R., Sallum, E. A., Casati, M. Z. & Nociti, F. H. Jr. (2007) A systematic review of stress and psychological factors as possible risk factors for periodontal disease. *Journal of Periodontology* **78**, 1491–1504.

Ritsner, M., Gibel, A., Maayan, R., Ratner, Y., Ram, E., Modai, I. & Weizman, A. (2007) State and trait related predictors of serum

cortisol to DHEA(S) molar ratios and hormone concentrations in schizophrenia patients. *European Neuropsychopharmacology* **17**, 257–264.

Shirayama, Y., Hashimoto, K., Suzuki, Y. & Higuchi, T. (2002) Correlation of plasma neurosteroid levels to the severity of negative symptoms in male patients with schizophrenia. *Schizophrenia Research* **58**, 69–74.

Snyder, D. S. & Unanue, E. R. (1982) Corticosteroids inhibit murine macrophage Ia expression and interleukin 1 production. *Journal of Immunology* **129**, 1803–1805.

Steptoe, A. & Ussher, M. (2006) Smoking, cortisol and nicotine. *International Journal of Psychophysiology* **59**, 228–235.

Tomar, S. L. & Asma, S. (2000) Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. *Journal of Periodontology* **71**, 743–751.

Williams, T. J. & Yarwood, H. (1990) Effect of glucocorticosteroids on microvascular permeability. *The American Review of Respiratory Disease* **141**, S39–S43.

Young, A. H., Gallagher, P. & Porter, R. J. (2002) Elevation of the cortisol-dehydroepiandrosterone ratio in drug-free depressed patients. *American Journal of Psychiatry* **159**, 1237–1239.

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Clinical Relevance

Scientific rationale for the study: A recently published systematic review suggested an association between psychological factors and periodontitis. However, the relationship between periodontitis and the stress-related hormones cortisol and DHEAS is poorly understood.

Principal findings: We found a close relationship between cortisol levels, including cortisol/DHEAS ratio, and periodontal status in subjects who had never smoked. Our findings suggest that these biomarkers are useful candidates for evaluating a part of the etiopathogenesis of periodontitis, at least for individuals who had never smoked.

Practical implications: There are several problems with conventional probing methods and exploration of biomarkers may open new avenues for development of a screening test for periodontitis in the near future.