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Evaluation of Psychosomatic Stress in Elective Spine Surgery by Measurement of Salivary Chromogranin A

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ABSTRACT

Introduction: Physical stress, such as operative time, blood loss, surgical wound size, and postoperative pain, is considered to be surgical stress. However, anxiety about and fear of surgery are also important elements of mental stress in surgery and are factors that may delay postoperative recovery. We determined changes in six test markers after spinal surgery and examined their associations with mental and physical stress-related items to evaluate the clinical usefulness of those markers.

Methods: Salivary chromogranin A (CgA) and five other markers (C-reactive protein (CRP), white blood cells (WBC) and interleukin-6 (IL-6) in serum, and amylase (Amy) and cortisol (Cor) in saliva) were measured in 46 patients before elective spine surgery and on postoperative days 1 and 7. At the same time, mental traits and psychological state were evaluated using mental stress-related items on the State-Trait Anxiety Inventory (STAI), a psychological stress test. Physical stress-related items of visual analog scale for pain, operative time, blood loss, and muscle invasiveness were also evaluated. The relationships of changes in six markers from pre- to post-surgery with the physical and mental stress-related items were examined using univariate and multivariate analyses.

Results: Changes in CRP, IL-6, and Cor after surgery were associated with physical stress. There was only a weak association of changes in CgA with mental stress (postoperative anxiety).

Conclusions: Measurement of changes in salivary CgA after surgery may be useful for objective evaluation of mental stress in the perioperative period of spinal surgery, and this warrants further study.

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KEYWORDS: perioperative stress, mental stress, spine surgery, chromogranin A, minimally invasive surgery

Introduction

Physical stress, such as operative time, blood loss, and postoperative pain, are key factors in surgical stress, but

mental stress is also important. Anxiety and tension with regard to surgery become mental stress. Such perioperative mental stress affects the autonomic nervous system and the immune system, thus influencing healing and post-

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Table 1 Characteristics of the patients

Age, median years (range)		65 (20, 84)
Sex	Men	30
	Women	16
Operation time, median minutes (range)		96 (53, 348)
Bleeding, median ml (range)		22.5 (0, 890)
Invasion of muscles	High	16
	Low	30

operative outcomes.¹⁾ Quantitation of the operative time, blood loss, postoperative pain, and serum cytokines has been used to evaluate physical stress in the perioperative period of spinal surgery,^{2,3)} but mental stress in this surgery has not been examined.

In this study, we focused on salivary chromogranin A (CgA) as a potential marker for mental stress. CgA is a biological index of stress that reflects the action of the sympathetic adrenomedullary system in the biological response to stress⁴⁾ and is released into saliva in response to autonomic nerve stimulation. Cortisol (Cor) is also a biological stress index,⁴⁾ but more significantly responds to physical stress, whereas CgA has characteristics of a poor response to physical stress but a good response to mental stress.⁵⁾ The CgA level may vary in blood due to pain and fear accompanying blood sampling, but mental stress can be accurately evaluated based on salivary CgA because there is no pain or fear accompanying sampling of saliva. Amylase (Amy) and Cor, which are conventional physical stress markers, were simultaneously measured in the collected saliva, and blood C-reactive protein (CRP), white blood cells (WBC), and interleukin-6 (IL-6) were also measured. Changes after spinal surgery for CgA CRP, WBC, AMY, IL-6, and Cor were determined, and elements of mental and physical stress associated with these changes were investigated to verify the clinical utility of the markers.

Methods

Subjects

Elective spine surgeries performed between June 2015 and December 2016 at our hospital were included in the study. Infection, trauma, malignant tumor, and reoperation cases were excluded, and only patients who gave written consent were included as subjects. Thus, subjects were 46

patients (males: 30, females: 16) who underwent surgery for spinal degenerative disease (Table 1). Age at the time of surgery was 20-84 years (mean age: 60.4 years). The median operative time and blood loss were 96 min and 22.5 ml, respectively. The target disease was cervical disk herniation in 2 patients, cervical spondylotic myelopathy in 5, lumbar disk herniation in 14, and lumbar spinal canal stenosis in 25. The patients were divided into two groups based on the surgical procedure: a low group (group L), comprising 16 cases in which the surgical procedure was slightly muscle-invasive and the patients were able to ambulate on the first hospital day, and a high group (group H), comprising 30 cases in which the procedure was relatively muscle-invasive and the patients ambulated on the second hospital day. Endoscopic nucleotomy followed by anterior cervical spinal fusion was performed in the 16 patients in group L; whereas posterior spinal fusion, fenestration, and cervical laminoplasty were performed in the 30 patients in group H. The pathology, surgical procedure, and treatment after surgery were explained to all patients before surgery. All surgeries were performed under general anesthesia, and pain relief by intravenous patient-controlled analgesia (IV-PCA) with fentanyl was performed for 24 hours after surgery. After this period, an NSAIDS was taken orally for 1 week. The study was performed after approval by the Ethics Committee of Toho University Omori Medical Center (27-35). The clinical study was explained to the subjects, and written consent was obtained from those who consented.

Physical and mental stress-related survey items

Operative time (min), blood loss (ml), and muscle invasiveness (groups L and H) were evaluated as physical surgical stress-related items, in addition to sex and age. A visual analog scale (VAS) was used only for wound pain

evaluation on the day before surgery and the first and seventh hospital days. Mental stress-related items were examined on the same days using the State-Trait Anxiety Inventory-Form JYZ (STAI) questionnaire as a psychological stress test.⁶⁾ STAI was used because anxiety as a state and anxiety due to personality traits can be measured simply. The STAI has two scales: state-anxiety STAI (s-STAI) for anxiety as a state and trait-anxiety STAI (t-STAI) for anxiety as a personality trait.⁷⁾

Test markers

Based on preceding studies,²⁻⁵⁾ CgA, CRP, WBC, Amy, IL-6, and Cor were used as biochemical markers of stress. Saliva was collected on the day before surgery and the first and seventh hospital days for measurement of salivary CgA, Amy, and Cor. Blood was collected after saliva sampling for measurement of serum CRP, WBC, and IL-6. Saliva was collected using the method reported by Nagasawa et al.⁸⁾ Patients chewed cotton for approximately 50 s to allow sufficient infiltration of the cotton by saliva. The salivary CgA level was measured using Salivette[®] for saliva (Sarstedt, Germany) with a YK070 Human Chromogranin A EIA kit (Yanaihara Institute Inc., Japan). The CgA concentration corrected with protein measured using a Bradford assay (pmol/mg protein) was used in analyses. Saliva was collected between 7:00 a.m. and 8:00 a.m. to exclude an effect of diurnal variation of CgA and prior to blood sampling to exclude the influence of a response on the mental stress of blood sampling.

Statistical analysis

Differences between the level of each marker on the day before surgery with those on the first and seventh hospital days were defined as $\Delta 0-1$ and $\Delta 0-7$ (CgA, CRP, WBC, Amy, IL-6, and Cor), and these were regarded as objective variables. In analyses of the differences between the day before surgery and the first hospital day ($\Delta 0-1$), s-STAI, t-STAI, VAS on day 1 (State1, Trait1, and VAS1), age, sex (men as reference), operative time, blood loss, and muscle invasiveness (group L as reference) were used as explanatory variables. In analyses of the differences between the day before surgery and the seventh hospital day ($\Delta 0-7$), s-STAI, t-STAI, VAS on day 7 (State7, Trait7, and VAS7) were used in addition to age, sex, operative time, blood loss and muscle invasiveness. The relationship of each objective variable with the explanatory variables was investigated by a univariate regression analysis, followed by a multiple-regression analysis, and is presented as the regression coefficient and its 95% confidence interval. Since

the correlation between operative time and blood loss was high ($r=0.7$), only operative time was included in multivariate analysis to avoid multicollinearity. No remarkable change occurred when blood loss was included in the models instead of operative time. All analyses were performed using Stata v.15 (StataCorp., College Station, TX, USA). Because the sample size was small, $p<0.05$ was regarded as statistically significant.

Results

Distribution of six markers

The levels of CgA, CRP, WBC, Amy, IL-6, and Cor on the day before surgery and first and seventh hospital days are given in Table 2. The highest CgA, WBC, Amy, and IL-6 levels occurred on day 1, whereas those for CRP and Cor were found on day 7. There was no sex difference in the levels of all markers before surgery.

State and trait-anxiety scores and the visual analog score

The values of s-STAI, t-STAI, and VAS at the three time points are listed in Table 3. The highest value of s-STAI occurred before surgery, whereas those of t-STAI and VAS were found on day 1.

Univariate and multivariate analysis for the difference in markers between pre-operation and 1 day after operation

The results of univariate and multivariate analyses of State1, Trait1, and VAS1, age, sex, operative time, blood loss, and muscle invasiveness for differences in the six markers between the day before surgery and the first hospital day are presented in Table 4. For Δ CgA 0-1, there was only a marginal association with State1 in the multivariate analysis ($p=0.097$) but not with any physical stress markers. For the other five markers, associations with Vas 1, age, sex, operative time, and muscle invasiveness were found in the univariate analysis. In the multivariate analysis, Δ CRP 0-1, Δ IL-6 0-1, and Δ Cor 0-1 were all associated with VAS1 and age; Δ WBC 0-1 was associated with age only, and Δ AMY 0-1 had no association. Δ CRP 0-1 and muscle invasiveness were associated in the multivariate analysis, but the change in CRP was smaller in group H than that in group L.

Univariate and multivariate analyses for the difference in markers between pre-operation and 7 days after operation

Results of univariate and multivariate analyses for changes in the six markers between the day before sur-

Table 2 Distribution of six markers at pre-operation and 1 day and 7 days after operation

	Pre-operation	1 day after operation	7 days after operation	Difference between pre-operation and 1day after operation	Difference between pre-operation and 7days after operation
	Median (25th percentile, 75th percentile)	Median (25th percentile, 75th percentile)	Median (25th percentile, 75th percentile)	Median (25th percentile, 75th percentile)	Median (25th percentile, 75th percentile)
CgA (pmol/mL)	3.3 (1.9, 6.7)	3.6 (2.1, 7.4)	3.5 (1.4, 6.2)	0.2 (- 2.4, 3.2)	0.2 (- 1.6, 3.5)
CRP (mg/dl)	0.1 (0, 0.2)	0.4 (0.1, 0.9)	0.6 (0.2, 1.9)	0.3 (0.1, 0.7)	0.5 (0.1, 1.7)
WBC ($\times 10^3/\mu\text{L}$)	6,050 (5,400, 7,200)	10,350 (8,400, 13,100)	6,250 (5,300, 8,700)	4,250 (2,500, 6,100)	500 (- 400, 1,400)
Amy (U/L)	108,900 (59,500, 228,000)	166,300 (51,000, 371,800)	54,600 (36,500, 122,400)	14,535 (- 41,771, 164,220)	- 35,105 (- 117,000, - 1,530)
IL-6 (pg/mL)	1.4 (0.9, 2.7)	11.2 (3.3, 18.8)	3.3 (1.7, 6.1)	8.3 (2.2, 17.9)	1.4 (0.2, 3.5)
Cortisol ($\mu\text{g/dL}$)	0.13 (0.08, 0.20)	0.06 (0.06, 0.26)	0.18 (0.13, 0.31)	- 0.02 (- 0.08, 0.15)	0.06 (- 0.03, 0.15)

Table 3 State and trait-anxiety scores and the visual analog score at pre-operation and 1 day and 7 days after operation

	Pre-operation	1 day after operation	7 days after operation
	Median (25th percentile, 75th percentile)	Median (25th percentile, 75th percentile)	Median (25th percentile, 75th percentile)
State	46 (38, 53)	43.5 (39, 52)	39.5 (32, 44)
Trait	41 (36, 50)	45 (36, 52)	42 (34, 48)
VAS	0 (0, 0)	4.5 (2, 5)	0 (0, 2)

gery and the seventh hospital day are listed in Table 5. In the univariate analysis, there was no association between ΔCgA 0-7 and State7. ΔCRP 0-7 and $\Delta\text{IL-6}$ 0-7 had associations with age, operative time, blood loss, and muscle invasiveness, and ΔCor 0-7 was associated with muscle invasiveness only. In the multivariate analysis, ΔCgA 0-7 was similarly not associated with State7; ΔCRP 0-7 was associated with age, sex, and operative time; and $\Delta\text{IL-6}$ 0-7 was associated with operative time. ΔCor 0-7 was associated with muscle invasiveness, but the change in Cor was smaller in group H than that in group L. Neither ΔCgA 0-1 nor ΔCgA 0-7 was associated with VAS for pain.

Discussion

Previous studies on spinal surgical stress have focused on so-called physical stress, such as operative time, blood loss, postoperative pain, and serum cytokines, especially IL-6.^{2,3)} By contrast, no study of spinal surgery-related mental stress has been reported. Kehlet proposed the fast-track concept in the 1990s.⁹⁾ This concept targets surgical

stress related to postoperative recovery and organ failure and refers to factors promoting and delaying postoperative recovery. The delaying factors before surgery include fear of and anxiety about surgery and lack of sleep; those during surgery include hypothermia and hypoxia; and those after surgery include indwelled tubes, such as a drain and postoperative pain. Factors that promote recovery include information on surgery and counseling, appropriate pain relief, early ambulation, and minimally invasive surgery. It has been found that postoperative recovery is promoted, and hospitalization is shortened by introducing a fast-track surgical program.¹⁰⁾ Regarding mental stress, approaches to psychological intervention have been investigated, such as calming, self-talking, and mental relaxation techniques.¹¹⁻¹⁴⁾

In this study, salivary CgA was investigated as a marker for objective evaluation of mental stress measured in a psychological stress test, STAI. Mental stress including personality was evaluated using t-STAI, and mental state was examined using s-STAI because there are indi-

Table 4 Univariate and multivariate analyses for the difference in markers between pre-operation and 1 day after operation

	ΔCgA 0-1		ΔCRP 0-1		ΔWBC 0-1		ΔAMY 0-1		ΔIL6 0-1		Δcor 0-1	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Univariate analysis												
Statel	0.09 (-0.02, 0.19)	0.112	0.02 (-0.01, 0.05)	0.169	-16.0 (-95.5, 63.5)	0.688	827.4 (-7,339.7, 8,994.5)	0.839	0.18 (-0.49, 0.85)	0.591	-0.001 (-0.007, 0.005)	0.772
Trait1	0.002 (-0.10, 0.11)	0.963	0.02 (-0.01, 0.05)	0.148	5.9 (-69.4, 81.3)	0.875	1207.5 (-6,513.8, 8,928.8)	0.754	0.13 (-0.51, 0.77)	0.685	-0.003 (-0.009, 0.002)	0.237
Vas1	0.21 (-0.29, 0.70)	0.409	0.21 (0.07, 0.34)	0.003 *	-101.7 (-461.5, 258.0)	0.572	8,398.9 (-28,550.3, 45,348.1)	0.649	4.2 (1.4, 7.0)	0.004 *	0.03 (-0.001, 0.05)	0.050
Age (year)	-0.03 (-0.10, 0.04)	0.364	0.02 (0.004, 0.05)	0.016 *	-66.6 (-114.8, -18.4)	0.008 *	4,659.5 (-51.2, 9,830.8)	0.076	0.6 (0.2, 1.0)	0.01 *	0.003 (-0.001, 0.006)	0.106
Sex (ref. men)	-0.38 (-2.69, 1.93)	0.740	0.44 (-0.23, 1.10)	0.193	-1,369.6 (-2,981.6, 242.4)	0.094	130,959.0 (-35,107.4, 297,025.3)	0.119	2.8 (-11.4, 16.9)	0.697	-0.06 (-0.19, 0.07)	0.338
Time (minute)	-0.01 (-0.03, 0.01)	0.360	0.001 (-0.005, 0.005)	0.988	10.9 (-1.4, 23.1)	0.079	799.4 (-473.8, 2,072.5)	0.212	0.1 (-0.1, 0.2)	0.552	-0.001 (-0.001, 0.001)	0.526
Bleeding (ml)	-0.001 (-0.007, 0.004)	0.672	0.001 (-0.002, 0.002)	0.998	-0.1 (-4.5, 4.4)	0.98	255.4 (-193.4, 704.1)	0.258	0.1 (-0.1, 0.1)	0.207	-0.001 (-0.001, 0.001)	0.827
Invasion (ref. low group)	-1.81 (-4.05, 0.44)	0.112	0.39 (-0.29, 1.06)	0.255	-221.3 (-1,884.7, 1,442.2)	0.79	72,043.8 (-97,314, 241,401.6)	0.396	16.4 (3.2, 29.6)	0.016 *	0.07 (-0.06, 0.19)	0.284
Multivariate analysis **												
Statel	0.12 (-0.02, 0.27)	0.097	0.02 (-0.02, 0.06)	0.317	-72.2 (-167.2, 22.8)	0.132	2,014.8 (-8,904.8, 12,934.5)	0.711	0.29 (-0.53, 1.11)	0.477	0.003 (-0.005, 0.010)	0.441
Trait1	-0.07 (-0.20, 0.07)	0.315	0.01 (-0.03, 0.04)	0.662	42.7 (-44.1, 129.5)	0.325	43.7 (-9,930.3, 10,017.8)	0.993	-0.17 (-0.92, 0.58)	0.650	-0.006 (-0.012, 0.001)	0.104
Vas1	0.34 (-0.21, 0.90)	0.22	0.22 (0.08, 0.36)	0.003 *	-173.9 (-532.7, 185.0)	0.333	6,362.0 (-34,880.6, 47,604.5)	0.757	3.84 (0.75, 6.95)	0.016 *	0.03 (0.003, 0.06)	0.031 *
Age (year)	0.03 (-0.06, 0.12)	0.525	0.04 (0.01, 0.06)	0.002 *	-96.8 (-156.8, -36.8)	0.002 *	5,541.5 (-1,357.4, 12,440.4)	0.112	0.56 (0.04, 1.08)	0.035 *	0.004 (-0.001, 0.008)	0.091
Sex (ref. men)	-0.50 (-2.85, 1.85)	0.669	0.22 (-0.37, 0.82)	0.454	-723.3 (-2,243.8, 797.2)	0.342	126,307.1 (-48,451.0, 301,065.2)	0.152	-0.78 (-13.9, 12.4)	0.905	-0.09 (-0.21, 0.03)	0.133
Time (minute)	-0.01 (-0.02, 0.01)	0.566	0.001 (-0.004, 0.005)	0.976	9.7 (-2.4, 21.9)	0.114	1,041.2 (-357.3, 2,439.7)	0.140	-0.002 (-0.10, 0.10)	0.963	-0.001 (-0.002, 0.0003)	0.199
Invasion (ref. low group)	-2.41 (-5.62, 0.79)	0.136	-0.69 (-1.50, 0.12)	0.093	1,266.0 (-812.6, 3,344.6)	0.225	-90,546.1 (-329,450.1, 148,358.0)	0.448	0.60 (-17.4, 18.6)	0.947	-0.01 (-0.17, 0.15)	0.906

* p<0.05

** Correlation between time and bleeding was high (r=0.7); therefore, only time was included in the multivariate analysis to avoid multicollinearity. Inclusion of bleeding in the models, instead of time, gave us a similar result.

Table 5 Univariate and multivariate analyses for the difference in markers between pre-operation and 7 days after operation

	ΔCgA 0-7		ΔCRP 0-7		ΔWBC 0-7		ΔAMY 0-7		ΔIL6 0-7		ΔCor 0-7	
	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Univariate analysis												
State7	- 0.003 (- 0.17, 0.17)	0.971	- 0.02 (- 0.09, 0.05)	0.569	17.9 (- 40.9, 76.8)	0.542	353.1 (- 3.208.5, 3.914.6)	0.843	0.03 (- 0.13, 0.18)	0.729	0.003 (- 0.005, 0.01)	0.435
Trait7	- 0.03 (- 0.16, 0.1)	0.622	- 0.02 (- 0.07, 0.03)	0.454	29.5 (- 15.2, 74.2)	0.19	395.2 (- 2.348.7, 3.138.9)	0.773	0.01 (- 0.11, 0.13)	0.891	0.001 (- 0.005, 0.007)	0.707
Vas7	- 0.17 (- 1.1, - 0.77)	0.719	0.20 (- 0.16, 0.55)	0.274	42.8 (- 292, 377.5)	0.798	11.481.0 (- 8.421.0, 31.383.0)	0.251	0.23 (- 0.61, 1.06)	0.583	- 0.014 (- 0.052, 0.025)	0.47
Age (year)	- 0.09 (- 0.18, 0.01)	0.064	0.05 (0.02, 0.09)	0.005 *	- 23.1 (- 56.1, 10.1)	0.167	- 782.8 (- 2.806.5, 1.241.1)	0.44	0.09 (- 0.01, 0.17)	0.051	- 0.001 (- 0.005, 0.003)	0.659
Sex (ref. men)	- 0.68 (- 3.66, 2.3)	0.648	- 0.86 (- 1.97, 0.26)	0.128	- 1.159.6 (- 2.176.3, - 143.0)	0.026 *	- 2.513.5 (- 67.407.6, 62.380.7)	0.938	- 0.49 (- 3.16, 2.2)	0.718	0.007 (- 0.116, 0.129)	0.919
Time (minute)	- 0.003 (- 0.03, 0.02)	0.823	0.01 (0.003, 0.02)	0.007 *	5.3 (- 2.8, 13.3)	0.196	- 4.0 (- 496.6, 488.6)	0.987	0.03 (0.01, 0.05)	0.004 *	- 0.001 (- 0.002, 0.001)	0.322
Bleeding (ml)	- 0.003 (- 0.01, 0.004)	0.379	0.003 (0.001, 0.006)	0.006 *	0.7 (- 2.3, 3.5)	0.661	- 91.6 (- 262.4, 79.3)	0.286	0.013 (0.007, 0.02)	0.001 *	- 0.001 (- 0.001, 0.001)	0.262
Invasion (ref. low group)	- 1.3 (- 4.3, 1.7)	0.391	1.3 (0.2, 2.4)	0.022 *	- 28.8 (- 1.104.7, 1.047.2)	0.957	- 28.156.1 (- 92.488.5, 36.176.3)	0.383	3.02 (0.5, 5.54)	0.02 *	- 0.133 (- 0.248, - 0.018)	0.025 *
Multivariate analysis **												
State7	0.08 (- 0.17, 0.33)	0.504	- 0.02 (- 0.09, 0.07)	0.723	- 9.0 (- 94.1, 76.2)	0.833	257.3 (- 5.212.7, 5.727.2)	0.925	- 0.001 (- 0.21, 0.21)	0.999	0.006 (- 0.005, 0.016)	0.277
Trait7	- 0.09 (- 0.28, 0.10)	0.331	- 0.007 (- 0.07, 0.06)	0.826	30.5 (- 34.9, 95.7)	0.351	260.9 (- 3.931.4, 4.453.2)	0.900	0.01 (- 0.15, 0.16)	0.947	- 0.001 (- 0.009, 0.007)	0.827
Vas7	- 0.05 (- 1.12, 1.03)	0.934	- 0.005 (- 0.35, 0.34)	0.978	14.2 (- 354.7, 383.1)	0.938	17.651.2 (- 6.047.7, 41.350)	0.140	- 0.42 (- 1.29, 0.47)	0.346	0.004 (- 0.039, 0.046)	0.865
Age (year)	- 0.10 (- 0.22, 0.02)	0.101	0.05 (0.009, 0.09)	0.019 *	- 19.3 (- 60.8, 22.3)	0.354	- 232.7 (- 2.899.8, 2.434.6)	0.861	0.06 (- 0.05, 0.16)	0.242	0.003 (- 0.003, 0.008)	0.296
Sex (ref. men)	- 0.32 (- 3.53, 2.89)	0.842	- 0.89 (- 1.92, 0.14)	0.087	- 983.2 (- 2.080.9, 1.14.7)	0.078	- 4.660.3 (- 75.184.4, 65.864.0)	0.894	- 0.21 (- 2.82, 2.41)	0.874	- 0.01 (- 0.136, 0.117)	0.880
Time (minute)	- 0.003 (- 0.029, 0.025)	0.858	0.01 (0.001, 0.02)	0.033 *	3.6 (- 5.5, 12.7)	0.428	- 55.8 (- 637.4, 526.0)	0.847	0.03 (0.01, 0.05)	0.017 *	- 0.001 (- 0.002, 0.001)	0.845
Invasion (ref. low group)	0.65 (- 3.53, 4.83)	0.755	0.14 (- 1.20, 1.47)	0.843	147.8 (- 1.282.9, 1.578.5)	0.836	- 44.068.5 (- 135.979.9, 47.843.0)	0.338	1.29 (- 2.11, 4.69)	0.448	- 0.193 (- 0.356, - 0.029)	0.022 *

* p < 0.05

** Correlation between time and bleeding was high (r = 0.7); therefore, only time was included in the multivariate analysis to avoid multicollinearity. Inclusion of bleeding in the models, instead of time, gave us a similar result.

vidual differences in stress sensitivity. In addition, differences in the salivary CgA level between the day before surgery and the first ($\Delta 0-1$) and 7th ($\Delta 0-7$) hospital days were investigated because there is no normal level of CgA, and individual differences are present in measurements before surgery.

When under stress, two stress response systems, the sympathetic nerve-adrenal medulla system and the hypothalamus-pituitary-adrenal cortex system, are activated, and catecholamines and Cor are secreted as stress hormones, respectively. The reaction of catecholamines to stress is higher than that of Cor, and catecholamines are considered appropriate for early detection of weak stress. However, normally, catecholamines are measured in blood or urine, and variation of the level due to pain and fear accompanying blood sampling is problematic, whereas urine sampling is difficult after surgery because a balloon is inserted. By contrast, Cor can be collected from saliva and is thus often used for stress evaluation, despite its lower stress responsiveness.⁴⁾

CgA is a glycoprotein that is associated with catecholamine storage and secretion.³⁾ CgA can be measured in saliva, and its secretion increases sharply and specifically in response to mental stress.¹⁵⁻¹⁸⁾ Secretion of salivary CgA has been found to increase with increased mental stress due to speech¹⁶⁾ and a cognitive task¹⁷⁾ but not with increased physical stress, such as ergometer exercise; therefore, CgA is thought to respond specifically to mental stress and is unlikely to be influenced by physical stress. CgA reaches its highest level of the day at waking and then maintains a lower level thereafter,²⁰⁾ making it important to consider diurnal variation in measurement. Thus, in this study, saliva was collected at a specified time point before blood sampling.

Studies on surgery-related salivary CgA are limited. Nakamura et al.²¹⁾ measured CgA in patients who received radiotherapy after breast-conserving surgery and found no significant correlation between STAI and salivary CgA at any time point from initiation to completion of radiotherapy and at 1 and 3 months after completion. We evaluated CgA with regard to perioperative mental stress of spinal surgery for the first time as far as we know. In this study, the association of Δ CgA 0-1 with State1 was marginal in the univariate analysis ($p=0.112$) and in the multivariate analysis ($p=0.097$), indicating only a weak association. However, Δ Cg 0-7 was not associated with State7 in the univariate or multivariate analysis. In addition, CgA was

not associated with VAS for postoperative pain or other physical stress-related items on the first or seventh hospital day. These findings suggest that CgA is not influenced by physical stress, including postoperative pain, but is influenced by mental stress, especially anxiety early after surgery. In addition, no association with sex or age was found, suggesting that CgA is a clinical marker that can be used regardless of these patient characteristics.

In multivariate analysis, Δ CRP 0-1, Δ IL-6 0-1, and Δ Cor 0-1 were associated with VAS1. IV-PCA with fentanyl was used for postoperative pain relief, but it may be influenced by pain early after surgery. In addition, Δ CRP 0-7 and Δ IL-6 0-7 were associated with operative time. The relationships of CRP, IL-6, and Cor with physical stress-related items were as expected. Furthermore, these markers were associated with age, and Δ CRP 0-7 was associated with sex. Thus, when these markers are used in a clinical setting, their interpretation should take each patient's demographics into account.

There are some limitations of this study. Diverse surgical procedures were used; detailed secretory dynamics of CgA were not examined; and the sample size was 46, which caused the confidence intervals of the analytical results to be wide. Therefore, a larger-scale study is required to verify the results.

In conclusion, the results of this study suggest that changes in CgA are marginally associated with mental stress and postoperative anxiety, whereas changes in CRP, IL-6, and Cor are associated with physical stress. Evaluation of changes in the salivary CgA level after surgery may be useful for objective evaluation of mental stress in the perioperative period of spinal surgery, and this warrants further study.

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Authors' contribution: Takahashi H. and Hasegawa K. designed the study; Hasegawa K., Wada A., Fukutake K., Nakamura K. and Nishiwaki Y. performed the experiments and analyzed the data; Takahashi K. supervised the experiments; Hasegawa K., Nishiwaki Y. and Takahashi H. wrote the manuscript.

Ethics statement: This study was conducted with the approval of the Ethics Committee of Toho University Omori Medical Center (27-35).

Conflicts of interest: None declared.

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