



Salivary Chromogranin A as a Psychosomatic Stress Marker Is Suppressed in Laparoscopic Surgery Compared with Open Surgery for Colon Cancer

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Objective: The advantages of laparoscopic surgery have been described in previous studies. The aim of this study was to objectively evaluate the benefits of laparoscopic surgery (LS) vs. conventional open surgery (OS) by measuring stress markers.

Materials: Fifty-four patients who underwent radical resection for primary colon cancer between May 2008 and March 2011 were enrolled. Thirty-two of the 54 patients underwent LS and twenty-two underwent OS.

Methods: Peripheral blood and saliva samples were obtained on five occasions during the perioperative period. Salivary Chromogranin A (CgA), derivatives of Reactive Oxygen Metabolite Test (d-ROMs Test), interleukin 6 (IL-6), natural killer (NK) cell activity and C-reactive protein (CRP) levels were analyzed for comparison between the two groups.

Results: With respect to the clinical characteristics, the intraoperative blood loss was significantly lower, and the operating time was longer in the LS group. Early postoperative oral-intake and reduced postoperative hospitalization were observed in the LS group. The salivary CgA, serum IL-6, and CRP were significantly lower in the LS group compared with the OS group. There was no difference in the d-ROMs finding between the two groups.

Conclusions: In the present study, there were significant differences in postoperative oral in-take, postoperative hospitalization, IL-6, CRP, and salivary CgA, suggesting an advantage of LS for patients with colon cancer. With respect to our finding of perioperative salivary CgA, further studies will be necessary to demonstrate the significance of this interesting and promising test.

Key words: chromogranin A (CgA), colon cancer, laparoscopic surgery (LS)

Introduction

There are many studies indicating the significance of laparoscopic surgery from various perspectives¹⁾⁻⁴⁾. However, few studies have previously evaluated the changes of stress markers in the perioperative periods^{5) 6)}. In this nonrandomized prospective study, we collected salivary and blood samples from patients with colon cancer during the perioperative periods, and compared parameters, such as stress markers and a cytokine, between the open surgery (OS) group and the laparoscopic

surgery (LS) group to objectively evaluate the relation between surgical stress and stress markers, and the benefits of laparoscopic procedures for patients with colon cancer.

Materials and Methods

This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee. All patients underwent surgical treatment at a single institution and provided written informed consent prior to enrolment in this study.

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Between May 2008 and March 2011, fifty-four of 57 patients, who underwent elective, radical resection for primary colon cancer with lymph node dissection were considered eligible. The patients decided to undergo either laparoscopic surgery or open surgery, after receiving sufficient explanations about advantages and disadvantages of laparoscopic surgery. Three of 57 patients were excluded from this study due to the intraoperative diagnoses of liver metastasis or peritoneal dissemination. Patients with synchronous or metachronous cancers, preoperative chemoradiation therapy, acute bowel obstruction or immune depressant disease or steroid treatment, inflammatory bowel disease, hemodialysis and severe medical illness were excluded from this study.

1. Surgical procedure

All procedures were performed by experienced colorectal surgeons at our institution. LS for colon cancer was conducted with five trocars under the lithotomy position. The peritoneal cavity was insufflated with carbon dioxide (CO₂), and pneumoperitoneum was maintained at an intra-abdominal pressure of eight to twelve mmHg. After early laparoscopic division of the large vessels and mobilization of the bowels with a medial-to-lateral approach, the specimen was exteriorized from 30 to 70 mm umbilical vertical using a midline minilaparotomy incision. OS was performed via a midline laparotomy incision ranging from 90 to 170 mm in length. In OS, a conventional lateral-to-medial approach was performed for mobilization of the bowels before division of the large vessels. Based on the Japanese classification of colorectal carcinoma⁷⁾, intestinal transection and D2 or D3 lymphadenectomy to the regional lymph nodes was performed for all procedures. Intestinal reconstruction was performed with end-to-end double-stapling technique to achieve anastomosis of the sigmoid colon and rectosigmoid cancer. For the others, functional end-to-end anastomosis with linear staplers or end-to-end, layer-by-layer hand-sewn anastomosis were selected arbitrarily, at the discretion of the operator.

2. Outcome parameters

Peripheral blood samples and salivary samples were obtained preoperatively, immediately after

surgery (postoperative days 0) and on postoperative days (POD) 1, 3, and 7. With respect to the stress indices, salivary chromogranin A (CgA) (pmol/ml) and derivatives of Reactive Oxygen Metabolite Test (d-ROMs Test) (U.CARR) were measured, in addition to conventional markers, i.e., serum interleukin-6 (IL-6) (pg/ml), natural killer (NK) cell activity (%), and C-reactive protein (CRP) (mg/dl).

Salivary CgA levels were measured using the YK070-Chromogranin A EIA kit (Yanaihara Institute Inc., Shizuoka, Japan). The levels of salivary CgA are 0.11 ± 0.05 pmol/ml in the morning^{8) 9)}.

The d-ROMs Test was performed to measure hydroperoxide using an automated analyzer, FRAS4 (Wismar Co. Ltd., Tokyo, Japan)¹⁰⁾. The results are expressed in arbitrary units, "U.CARR". Reference values from normal samples have been reported between 250 and 300 U.CARR; a U.CARR measurement of 300 or more would suggest a condition of oxidative stress, as a consequence of abnormal production of free radicals.

NK cell activity was measured by cytotoxicity test using the ⁵¹Cr-release method¹¹⁾. The reference value for the NK cell activity under normal conditions has been established as between 18 and 40%.

3. Statistical analysis

Statistical analyses were carried out using JMP® 10.0.2 (SAS Institute Inc., North Carolina, USA). Patient background data were compared using Fisher exact test, χ^2 test and Wilcoxon rank sum. Serial changes in the end-points were analyzed using repeated measures ANOVA with a mixed effect model and replication using group and time as variable factors. The probability curve of overall survival was constructed using the Kaplan-Meier method and the two groups were compared using the log-rank test. Differences were considered statistically significant at $p < 0.05$.

Results

With respect to the clinical characteristics, there were no differences in age, gender, tumor location, extent of lymph node dissection, the number of retrieved lymph nodes or the TNM stage (UICC^{7th}) between the two groups (Table-1). With respect to the TNM classifications, the LS group tended to include many early-stage patients, who underwent

Table-1 Clinical characteristics

		laparoscopic surgery group (n = 32)	open surgery group (n = 22)	p-value
Age, years		65.5 (44-80)	71.5 (55-87)	0.204
Sex, M/F		18/14	8/14	0.151
Tumor location*, n	C	3 (9.4%)	1 (4.6%)	0.964
	A	9 (28.1%)	6 (27.3%)	
	T	2 (6.2%)	2 (9.1%)	
	D	1 (3.1%)	1 (4.6%)	
	S	11 (34.5%)	9 (40.9%)	
	RS	6 (18.8%)	3 (13.6%)	
Lymph node dissection†, n	D2	5 (22.7%)	7 (21.9%)	0.941
	D3	17 (77.3%)	25 (78.1%)	
Number of retrieved lymph nodes, n		23.5 (11-41)	21.5 (14-65)	0.622
TNM stage (UICC 7 th), n	I	18 (56.3%)	6 (27.3%)	0.128
	II A	5 (15.6%)	7 (31.8%)	
	II B	2 (6.3%)	0	
	III A	1 (3.1%)	0	
	III B	4 (12.5%)	5 (22.7%)	
	III C	2 (6.3%)	4 (18.2%)	

* C: Cecum, A: Ascending colon, T: Transverse colon, D: Descending colon, S: Sigmoid colon, RS: rectosigmoid

† Japanese classification of colorectal carcinoma

Table-2 Operative and postoperative data

	laparoscopic surgery group (n = 32)	open surgery group (n = 22)	p-value
Duration of operation, min	266 (186-337)	231 (140-405)	p < 0.001
Blood loss, ml	40 (5-142)	150 (10-500)	p < 0.001
Time to begin oral intake, days	5 (4-10)	6 (4-20)	p < 0.001
Postoperative hospital stay, days	9 (8-30)	12 (9-32)	p < 0.001

Data are expressed as median (range)

surgery after endoscopic resection of the primary colon cancer.

The operative time was significantly longer and operative blood loss was significantly lower in the LS group compared with the OS group (Table-2) ($p < 0.001$). Both the time to initiating oral-intake and duration of the hospitalization following surgery were significantly shorter in the LS group compared with the OS group ($p < 0.001$).

Salivary CgA and serum IL-6 increased on POD0 and decreased gradually from POD1 (Figure-1A, B). CRP did not increase on POD0 but rather from POD1, and decreased from POD7 (Figure-1C).

The increases of salivary CgA, IL-6, and CRP levels in the LS group were significantly lower than those in the OS group ($p = 0.048$, $p < 0.001$, $p = 0.004$). In the d-ROMs test, the value decreased on POD0 and POD1, and subsequently increased above the baseline on POD3 and 7 (Figure-1D). The NK cell activity decreased transiently on POD1, and returned to baseline on POD3 (Figure-1E).

We assessed the long-term outcome, with a median follow-up period of 63 months (range: 9-83 months), and the probability of overall survival was not significantly different between the two groups (Figure-2).

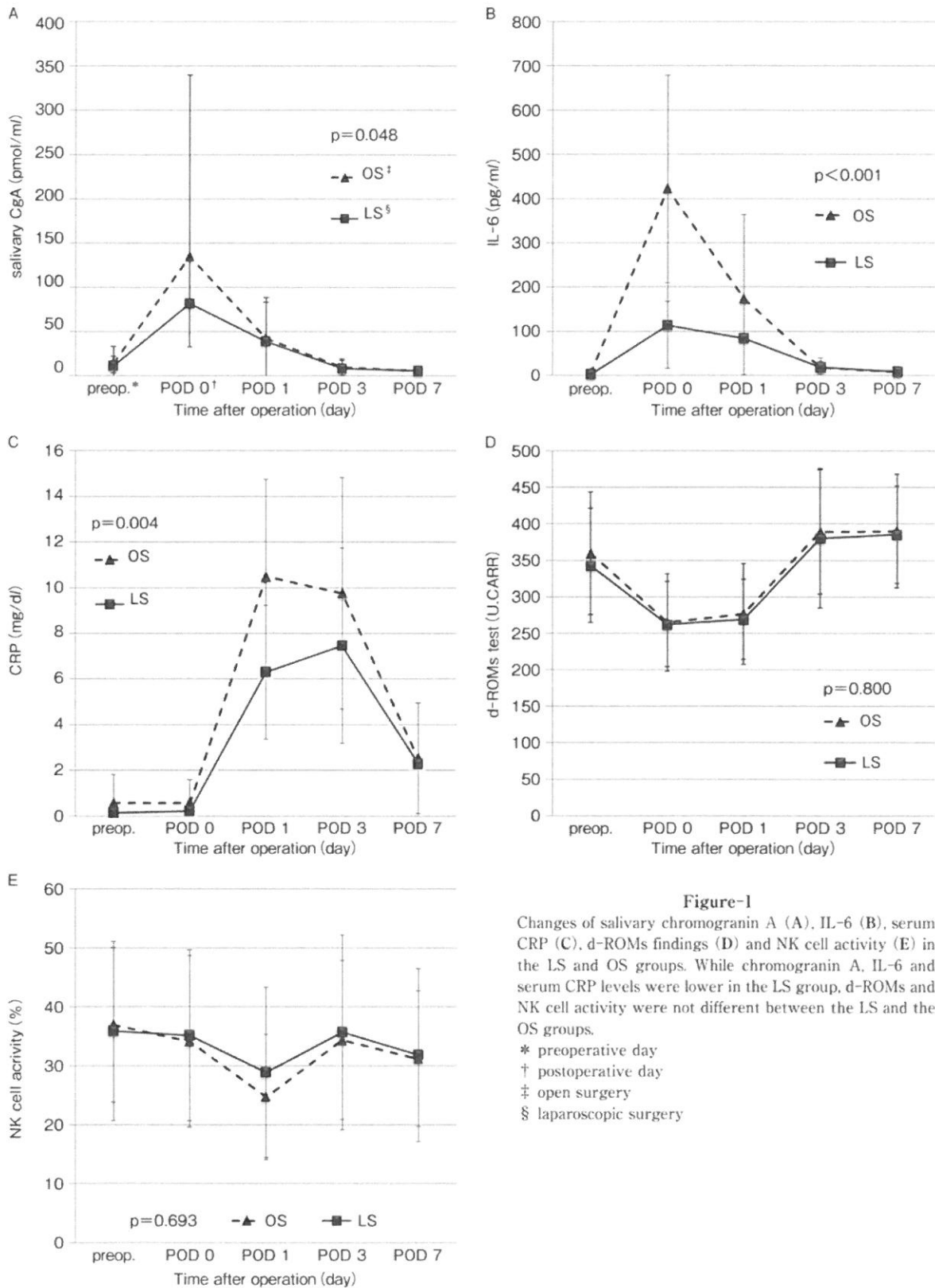


Figure-1

Changes of salivary chromogranin A (A), IL-6 (B), serum CRP (C), d-ROMs findings (D) and NK cell activity (E) in the LS and OS groups. While chromogranin A, IL-6 and serum CRP levels were lower in the LS group, d-ROMs and NK cell activity were not different between the LS and the OS groups.

* preoperative day
 † postoperative day
 ‡ open surgery
 § laparoscopic surgery

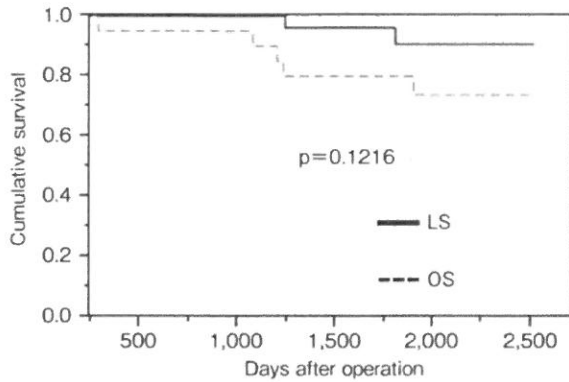


Figure-2 Comparison of the overall survival rate between the LS and OS groups

There was no significant difference in survival between the 2 groups.

Discussion

The first minimally invasive colon resection was described in 1991 by Jacobs *et al.*¹²⁾. Since then, it has been reported that the laparoscopic procedure is feasible in most patients with benign colonic diseases¹³⁾. It has also been reported that the laparoscopic approach for colon cancer led to improved postoperative recovery, and was equivalent with respect to complications and long-term outcomes^{1) 14) 15)}. More recently, laparoscopic surgery for colon cancer has increased remarkably in Japan¹⁶⁾, mainly because it is regarded as technically feasible due to standardization of the laparoscopic operative procedure through advances of stapling technology and energy delivery systems. In laparoscopic surgery, reductions in the bleeding, variation in the peritoneal environment, and abdominal injury support the minimally-invasiveness of the procedure. In comparison to open surgery, laparoscopic resection of colon cancer improved patient comfort due to its reduced surgical trauma. Reduced inflammation in the body and impact on immune function may lead to pain relief and early recovery after laparoscopic surgery¹⁷⁾.

Many studies have compared laparoscopic surgery with open surgery from a number of perspectives, and early postoperative recovery and suppression of inflammatory responses have almost uniformly been reported with LS¹⁾⁻⁴⁾. In the present study, LS was associated with early postoperative oral-intake, reduction of postoperative hospitalization, and suppression of the rise of IL-6 and CRP.

IL-6 and CRP are general indicators of inflammatory and immune responses. However, they can also increase following tissue injury in surgery^{18) 19)}. In several previous reports, IL-6 levels rose from a couple of hours after the initial incision and peaked at 4-48 hours (median: 8 hours)^{19) 20)}. Plasma CRP generally begins to increase from 6-8 hours after incision with the peak concentration of CRP occurring at 48 hours²¹⁾. It is widely known that IL-6 induces hepatic synthesis of CRP and other acute-phase proteins^{22) 23)}. Our data essentially suggested advantages of LS that were consistent with previous reports¹⁾⁻⁴⁾.

NK cell activity is considered to be one of the indices of cell-mediated immune response. The more severe the surgical stress is, the suppression of the NK cell activity should become hypothetically larger. However, postoperative suppression of NK cell activity have not been uniformly smaller in laparoscopic surgery in previous reports^{24) 25)}. There was no difference in NK cell activity between the two groups in this study. Small sample size and wide range of reference value might have affected the outcomes.

In the d-ROMs test, as a marker of oxidative stress, there was no difference between the two groups. Perioperative changes of d-ROMs have not been well-described to date⁵⁾. In our study, the values increased above the baseline at POD3 and 7, after an initial suppression at POD0 and 1. The changes of the values during the perioperative period could suggest that the oxidative stress was influenced by another factor, distinct from surgery. Because propofol, which is known to be a strong antioxidant⁶⁾, was used during anesthesia for all surgeries, this could have affected the results. Although some reports have suggested that propofol exhibits a strong antioxidant effect, details of the effects remain unknown. Further studies will be necessary to elucidate the influence of propofol administration during the postoperative period.

CgA is the major soluble protein that is produced by endocrine and neuroendocrine cells, and is co-stored and subsequently co-released along with catecholamines from the adrenal medulla and neuronal vesicles in response to external stimuli²⁶⁾. Acute stress increases the activity of the sympatho-adrenal medullary system and may promote secretion of CgA. Saliva might be a suitable sample for

stress research, since venipuncture is considered as a "potent psychological and physiological stressor"²⁷⁾. Levels of CgA increase rapidly in saliva after exposure to stress and decrease rapidly after the stress is alleviated; suggesting that it may be suitable in the evaluation of acute stress^{28) 29)}. Nakane *et al.* reported that salivary CgA concentrations increased rapidly under psychosomatic stress³⁰⁾. To our knowledge, there has been no previous evaluation of salivary CgA during the perioperative period. In the present study, the changes in salivary CgA following surgery showed significant suppression of the postoperative response in the LS group. We hypothesized that the laparoscopic procedure could result in some degree of reduced surgical or psychological stress. Because the salivary CgA test is simple enough and non-invasive as a stress marker, perioperative salivary CgA deserves further investigation to determine its utility for future use.

The probability of overall survival was not significantly different between the two surgical groups. However, a tendency towards better overall survival might be evident in the LS group, as many early-stage patients were included in the LS group.

The limitations of this study include its non-randomized, single-center design and small sample size.

In conclusion, there were significant differences in the postoperative oral in-take, postoperative duration of hospitalization, salivary CgA, IL-6, and CRP, suggesting the advantage of LS for patients with colon cancer. Regarding our finding of perioperative salivary CgA, further studies will be necessary to demonstrate the significance of this interesting and promising test.

Conflicts of interest

The authors declare no conflict of interest associated with this manuscript.

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