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Production of Intraperitoneal Interleukin-6 Following Open or Laparoscopic Assisted Distal Gastrectomy

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The interleukin (IL)-6 concentration in plasma or serum has been considered to represent the degree of stress resulting from surgery. However, IL-6 in peritoneal fluid has rarely been considered. The aim of this study was to assess the concentration and amount of IL-6 in peritoneal fluid as indicators of surgical stress. To obtain basic data on peritoneal release of IL-6 during gastric cancer surgery, we measured IL-6 in peritoneal drainage samples, stored for up to 72 hours postoperatively, from patients who had undergone conventional open (ODG group, n = 20) and laparoscopic-assisted (LADG group, n = 19) distal gastrectomy. Within 24 hours, 61 and 77% of the IL-6 was released into the peritoneal cavity in the LADG and ODG groups, respectively. In both groups, the concentration and amount of peritoneal fluid IL-6 were significantly correlated with each other (LADG group: Spearman's rank correlation test [rS] = 0.48, $P = 0.04$; ODG group: $rS = 0.58$, $P = 0.01$). The concentration and amount of IL-6 in peritoneal fluid was 2.8- and 3.6-fold higher in the ODG than in the LADG group, respectively ($P < 0.01$). With regard to the relationship between the serum C-reactive protein (CRP) peak and the concentration or amount of peritoneal fluid IL-6 released within 24 hours, only the concentration of peritoneal fluid IL-6 in the LADG group was significantly correlated ($rS = 0.60$, $P = 0.01$) with the serum CRP peak. Our findings suggest that the amount and concentration of IL-6 released into the peritoneal cavity for up to 24 hours after surgery can each be a reliable parameter for assessment of surgical stress.

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Recent advances in laparoscopy techniques have heralded a new era in the field of abdominal surgery, and laparoscopic approaches are now being employed for the treatment of malignant neoplasms of the stomach, colon and other organs.[1–5]

It has been reported that levels of circulating proinflammatory cytokines such as interleukin (IL)-1 beta, IL-6, IL-8, and tumor necrosis factor (TNF)-alpha are related to the extent and severity of surgical procedures.[6–8] Interleukin 6 is a sensitive and early marker of tissue damage, and in general the greater the surgical trauma, the greater the response of IL-6.[9] Many studies have shown that the level of IL-6, as an indicator of surgical stress, is significantly lower after laparoscopic surgery than after open surgery.[9–15] Most of the studies reported so far have measured and analyzed the concentration of IL-6 in plasma or serum,[9,10,12–16,18] and only rarely in peritoneal fluid,[11,12,15,17,19,20] and the results have indicated significant correlations between the level of IL-6 and several clinical parameters. However, there is some concern as to whether the concentration of IL-6 in peritoneal fluid adequately reflects the degree of surgical stress. Interleukin 6 is released into the abdominal cavity in response to surgical injury.[16,20,21] Therefore, the total amount of IL-6 contained in peritoneal fluid might be regarded as a gold standard for assessing the severity of local surgical stress. However, this possibility has never been fully addressed.

In the present study, we analyzed both the concentration and total amount of IL-6 in peritoneal fluid sequentially in the early postoperative period after distal gastrectomy, and compared laparoscopic and open surgical procedures in terms of intraperitoneal IL-6 production in order to observe the basic pattern of IL-6 release.

Materials and Methods

Written informed consent was obtained from all patients, and the study was performed under a protocol approved by our hospital ethics committee. The level of lymph node dissection and pathological stage were in accordance with the general guidelines for treatment of gastric cancer in Japan.[22,23]

Between January and December 2012, twenty consecutive patients who underwent elective open distal gastrectomy (ODG group) and 19 patients who underwent elective laparoscopic-assisted distal gastrectomy (LADG group) for gastric cancer at our single institution were prospectively enrolled for this study. Laparoscopic-assisted surgery was used for patients in whom the depth of tumor invasion had been assessed as limited to the submucosa before treatment. We routinely placed a low-vacuum JVAC drain (Ethicon, Inc, Bridgewater, NJ, USA) infrahepatically (near the resection site) for drainage of peritoneal fluid and removed it within 4 days after surgery. Peritoneal fluid was collected at 24, 48, and 72 hours after surgery. The samples were centrifuged for 10 minutes at 3000 rpm at 4°C and then stored in aliquots at −80°C until tested. Interleukin 6 levels were analyzed using a commercially available solid-phase sandwich enzyme-linked immunosorbent assay kit with a monoclonal antibody specific for IL-6 (R&D Systems Co, Minneapolis, MN, USA).

Using the collected samples of peritoneal fluid, we measured and calculated the following 2 parameters: the concentration of IL-6 (pg/mL) at 24, 48, and 72 hours after surgery and the amount of IL-6 (μg)—*i.e.*, the concentration of IL-6 multiplied by the volume of peritoneal fluid—at the same 3 time points. The total amount or concentration of IL-6 was expressed in relation to the timing of sample collection: phase I (up to 24 hours), phase II (24 ∼ 48 hours), and phase III (48 ∼ 72 hours). Serum levels of C-reactive protein were measured according to routine clinical practice on postoperative days (POD) 1, 4, and 7.

We also evaluated clinical parameters such as patient age and sex, operation time, intraoperative blood loss, perioperative complications, degree of lymph node dissection, and pathological stage.

Statistical Analysis

Variables were expressed as median and range. Spearman's rank correlation and Friedman tests were applied for examining correlations between variables. Differences between groups were analyzed by Mann-Whitney U test and χ^2 test. Differences at $P < 0.05$ were considered significant.

Table 1 Clinical characteristics and operative outcome

	Total	LADG group (n = 19)	ODG group (n = 20)	P value
Age	68.0 (41.0–86.0)	65.0 (48.0–83.0)	68.0 (41.0–86.0)	0.92
Sex				
Male	25	12	13	0.905
Female	14	7	7	
Operation time, min	185.0 (95.0–378.0)	202.0 (137.0–378.0)	178.5 (95.0–281.0)	0.052
Blood loss, mL	105.0 (5.0–740.0)	15.0 (5.0–145.0)	230.0 (345.0–740.0)	<0.01
Perioperative, +	3	1	2	0.58
Complication, –	36	18	18	
Lymph node dissection				
D1	16	14	2	<0.01
D1+	15	2	13	
D2	8	3	5	
Pathological stage				
1	20	16	4	<0.01
2	4	2	4	
3	8	1	7	
4	5	0	5	

Results

Patient background

Patients' characteristics and operative outcomes are shown in Table 1. There were no significant differences between the LADG and ODG groups in terms of age and sex (χ^2 test, $P = 0.92$ and 0.90, respectively). The OGD group underwent more extended lymph node dissection and had a more advanced pathological stage than the LADG group (χ^2 test, $P < 0.01$). The median operation time was 202 minutes in the LADG group and 178.5 minutes in the ODG group, and this difference was marginally significant (Mann-Whitney U test, $P = 0.052$). Blood loss was significantly less in the LADG group than in the DG group (Mann-Whitney U test, $P < 0.01$). Perioperative complications occurred in 3 of the 39 patients. In the LADG group, 1 patient developed leakage of pancreatic juice; and in the ODG group, 1 patient each developed wound infection and anastomotic leakage.

Table 2 Amount of peritoneal fluid and IL-6

	LADG group	ODG group	P value (Mann-Whitney U test)
Fluid amount, mL			
Phase I	110.0 (12.0–1120.0)**	195.0 (70.0–920.0)*	0.02
Phase II	70.0 (8.0–670.0)**	75.0 (1.0–360.0)*	0.55
Phase III	60.0 (3.0–780.0)**	63.5 (2.0–334.0)*	0.54
Total	358.8 (23.0–2570)	685.0 (91.0–1560.0)	0.11
Concentration of IL-6 ($\times 10^4$ pg/mL)			
Phase I	2.42 (0.97–35.33)**	6.81 (1.83–14.50)*	<0.01
Phase II	2.31 (0.28–16.91)**	3.44 (0.87–14.09)*	0.22
Phase III	1.88 (0.20–6.60)**	2.19 (0.10–10.20)*	0.60
Amount of IL-6 ($\times 10^6$ pg)			
Phase I	3.60× (0.55–14.00)*	13.0 (1.28–84.73)*	<0.01
Phase II	1.57 (0.17–7.95)*	2.71 (0.04–31.00)*	0.10
Phase III	0.53 (0.09–9.02)*	1.12 (0.01–21.44)*	0.47
Total	6.03 (2.01–19.95)	19.20 (2.22–97.71)	<0.01

*Friedman test: $P < 0.001$.

**$P < 0.01$.

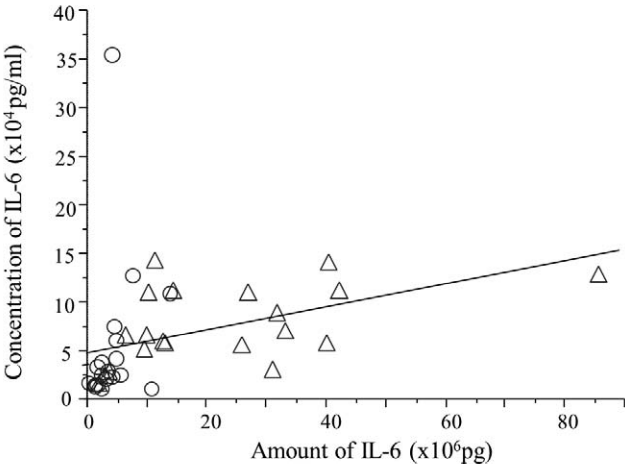

Fig. 1 Correlation between amount and concentration of IL-6 (phase I). Circular dots: laparoscopic assisted distal gastrectomy cases. Triangular dots: open distal gastrectomy cases. Spearman's rank correlation test: $rS = 0.68$, $P < 0.01$.

Characteristics of peritoneal fluid IL-6 (Table 2)

Regardless of the type of surgery (LADG or ODG), the amount of peritoneal fluid collected via the closed suction drain decreased significantly in the order phase I > phase II > phase III. The median volume of peritoneal fluid during phase I in the ODG group was 1.8-fold higher than that in the LADG group (110.0 mL versus 195.0 mL, $P = 0.02$). In phases II and III, however, there were no significant intergroup differences in the amount of peritoneal fluid.

Similarly, the concentration of IL-6 in the peritoneal fluid decreased significantly in the order phase I > phase II > phase III in both groups. During phase I, the concentration of IL-6 in peritoneal fluid was 2.8-fold higher in the ODG group than in the LADG group ($P < 0.01$). In phases II and III, however, the IL-6 concentration in peritoneal fluid did not differ significantly between the two groups ($P = 0.22$ for phase II and $P = 0.60$ for phase III).

The amount of IL-6 contained in the peritoneal fluid also decreased significantly in the order of phase I > phase II > phase III in both groups.

Table 3 Correlation between amount of IL-6 (phase I) and clinical parameters

	rS (P value)		
	LADG group	ODG group	Total
Blood loss (mL)	0.33 (0.17)	0.22 (0.34)	0.55 (<0.01)
Operation time (min)	0.26 (0.27)	0.16 (0.50)	–0.10 (0.55)

Table 4 Serum CRP level during perioperative period

	LADG group	ODG group
CRP (mg/dL)		
POD 1	4.8 (1.0–11.1)*	7.7 (2.1–15.5)*
POD 4	4.5 (1.0–21.3)*	8.2 (1.2–16.8)*
POD 7	1.5 (0.0–7.0)*	2.55 (0.5–17.4)*

*Friedman test: $P < 0.001$.

Again, during phase I, the amount of IL-6 in peritoneal fluid was 3.6-fold higher in the ODG group than in the LADG group ($P < 0.01$). In phases II and III, however, no such difference was evident between the groups ($P = 0.10$ for phase II and $P = 0.47$ for phase III).

The proportional amount of IL-6 in peritoneal fluid during phase I relative to that during the 3 phases as a whole was 61% (range, 17–82%) in the LADG group and 77% (range, 24–97%) in the ODG group.

When patients in the ODG and LADG groups were analyzed collectively, the correlation between the concentration and the amount of IL-6 during phase I was strongly significant ($rS = 0.68$, $P < 0.01$; Fig. 1). When analyzed for each group separately, the corresponding correlations were also significant, but weak ($rS = 0.48$, $P = 0.04$ in the LADG group, and $rS = 0.58$, $P = 0.01$ in the ODG group).

Correlation between the amount of IL-6 in phase I and operation time or blood loss (Table 3)

When both groups were analyzed collectively, blood loss showed a significant but weak correlation with the amount of IL-6 in phase I ($rS = 0.55$, $P < 0.01$). However, when the groups were analyzed separately, no such correlation was recognized. There was no significant correlation between the amount of IL-6 and operation time in any group.

Correlation between the peak serum CRP level and IL-6 in peritoneal fluid during phase I

In the ODG group, the peak serum CRP level occurred at POD 4, and the differences between PODs 1, 4, and 7 were significant (Friedman test, $P < 0.01$). In the LADG group, the peak serum CRP level occurred at POD 1, and the differences between PODs 1, 4, and 7 were significant (Friedman test, $P < 0.01$; Table 4).

In the ODG group, the correlation between the serum peak level of CRP (on POD 4) and the

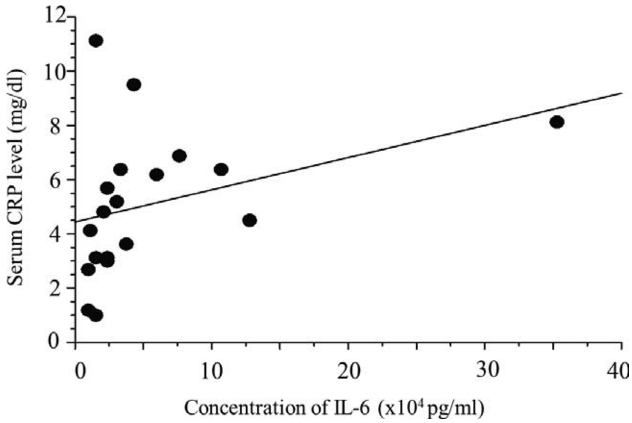

Fig. 2 Correlation between the serum CRP level (POD 1) and concentration of IL-6 (LADG case). Spearman's rank correlation test: $rS = 0.60$, $P = 0.01$.

concentration or amount of peritoneal fluid IL-6 in phase I was not significant ($rS = 0.20$, $P = 0.39$, $rS = 0.38$, $P = 13$, respectively). In the LADG group, the serum peak level of CRP (on POD 1) showed a significant but weak correlation with the concentration of peritoneal fluid IL-6 in phase I ($rS = 0.60$, $P = 0.01$; Fig. 2), but did not show any correlation with the amount of peritoneal IL-6 ($rS = 0.13$, $P = 0.53$).

Discussion

Surgery induces a complex inflammatory cascade, which is initiated by IL-1 and TNF-alpha, followed by IL-6 and IL-8.[6–8] These cytokines are released sequentially into the peritoneal cavity and the bloodstream following abdominal surgery,[16,20,21] and are important mediators of the inflammatory response at sites of injury or infection.[12] Among them, IL-6 is known to induce maturation of B-cell lymphocytes and synthesis of acute-phase proteins, including CRP in the liver.[24] An increased level of IL-6 in blood resulting from local production is considered to reflect the degree of surgical stress, and may produce a systemic immunologic effect.[21] Many cytokines, including IL-6, are reported to be produced by macrophages, peritoneal mesothelial cells, and polymorphonuclear leukocytes as a peritoneal response to surgical injury.[25,26] Thus, the cytokine concentrations in peritoneal fluid after major surgery are far higher than in blood, suggesting that cytokine production after gastrointestinal surgery occurs in a compartmentalized fashion within the abdominal cavity.[20]

The majority of previous studies have focused on the concentration of IL-6 in peritoneal fluid, and found that it was significantly correlated with a number of clinical parameters.[11,12,15,17,19,20] In addition to the concentration of IL-6, the absolute amount of IL-6 in peritoneal fluid has also been investigated for evaluation of surgical stress.[27] Although the concentration of IL-6 in serum or plasma is widely used to assess surgical stress, the blood level of IL-6 is reported to vary widely according to the timing of sample collection, and is thus considered difficult to apply clinically.[16] It still remains controversial which type of sample should be adopted for evaluating the level of IL-6. However, no reliable data for resolving this issue have been available.

In the present study, we collected the total amount of the peritoneal fluid drained until 3 days after surgery and evaluated both the concentration and absolute amount of IL-6 in the fluid for each of the 3 postoperative days. We found that approximately 80 and 60% of the total IL-6 was released into the abdominal cavity during the first 24 hours after surgery in the ODG and LADG groups, respectively. In addition, we found a strong correlation between the amount and the concentration of IL-6. These findings suggest that the amount or concentration of IL-6 released into the peritoneal cavity for up to 24 hours after surgery would be a reasonable and reliable parameter for assessment of surgical stress. Alternatively, for this purpose, it might be sufficient to simply evaluate the concentration, rather than the amount, of IL-6 in peritoneal fluid. These results lend support to the methods adopted in previous investigations[11,12,15,17,19,20] that measured and analyzed the concentration of IL-6 in peritoneal fluid, even though such investigations adopted a simple sampling procedure and did not collect peritoneal fluid that had accumulated during specific periods.

The concentration of IL-6 in peritoneal fluid collected up to 24 hours after surgery was 2.8-fold higher ($P < 0.01$) in the ODG group than in the LADG group. Our results seem to be inconsistent with those of Jung *et al*,[17] who found no significant difference in the concentration of IL-6 in peritoneal fluid between LADG and ODG groups at 24 hours after surgery. It was not our intention to directly compare pneumoperitoneum and open procedures, since some clinicopathological background factors and the level of lymph node dissection differed between the present surgical treatment groups. Rather, our aim was to obtain basic data on IL-6 released into the peritoneal cavity and to clarify the optimal methods of sample collection and analysis.

Our results revealed a significant correlation between IL-6 and blood loss during surgery when ODG and LADG patients were analyzed collectively. This may have been partly due to the fact that the LADG group showed a significantly lower amount of IL-6 and lower intraoperative blood loss than the ODG group, even though there was no significant positive correlation between the amount of IL-6 (phase I) and intraoperative blood loss in each group. However, when the 2 groups were analyzed separately, blood loss revealed no significant correlation with the amount of IL-6. Irrespective of whether the 2 groups were analyzed collectively or separately, there were no significant correlations between operation time and the concentration or amount of IL-6 in peritoneal fluid, in contrast with previous reports. Tsukada *et al*[19,28] reported a significant correlation between operation time and the concentration of IL-6 in peritoneal fluid at 24 hours after surgery. West *et al*[29] reported that peritoneal CO_2 insufflation decreased the amounts of TNFα and IL-1 released from murine macrophages in vitro, suggesting overall inhibition of IL-6 production. It would be interesting to investigate whether IL-6 production during laparoscopic surgery is affected by the duration of CO_2 insufflation. However, our data revealed no significant correlation between the duration of surgery and IL-6 production. As the number of LADG cases was limited in the present study, collection of further data would seem warranted.

In the present study, regardless of the type of surgical procedure (open or laparoscopic), the peak level of CRP in serum appeared to have no close correlation with intraperitoneal release of IL-6. Thus, measurement of IL-6 in peritoneal fluid in the early postoperative period (i.e. within 24 hours of surgery), might be more closely representative of the peritoneal response to intra-abdominal surgical stress than measurement of the blood CRP level. We believe that our present basic data will help to clarify the peritoneal response to various types of intra-abdominal surgery, including gastric cancer surgery.

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There are no conflicts of interest in relation to this manuscript.

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