

Comparison of Immune Preservation Between CO₂ Pneumoperitoneum and Gasless Abdominal Lift Laparoscopy

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ABSTRACT

Objective: Carbon dioxide (CO₂) pneumoperitoneum has been implicated as a possible factor in early immune preservation in laparoscopic surgery. Although the current analysis was not adequate to clarify this issue, the aim of this study was to compare CO₂ insufflation laparoscopic cholecystectomy to gasless abdominal wall lift laparoscopic cholecystectomy with respect to preservation of the immune system.

Method: An analysis of the temporal immune responses was performed in 2 similar groups of patients (n = 50) who were divided randomly into the categories of gas or abdominal wall lift laparoscopic cholecystectomy. The patients were matched with respect to age, weight, and operation time. The immune parameters (serum white blood cell count, cortisol, erythrocyte sedimentation rate [ESR], tumor necrosis factor- α [TNF- α], interferon- γ [INF- γ], interleukin-6 [IL-6], interleukin-8 [IL-8]) were assessed at preoperative 24 hours and at postoperative 24 and 72 hours for the 2 groups. During the operation, the levels of cytokines that were cultured in the peritoneal macrophages were also checked.

Results: The serum white blood cell count, cortisol, and ESR levels were not statistically different in either of the 2 groups. Further, the serum TNF- α , INF- γ , IL-6, and IL-8 levels in both groups were not significantly different from each other at preoperative 24 hours, and postoperative 24 and 72 hours. However, an immediate decrease in the cytokine levels at 24 hours after the operation was significant in both groups. The cytokine levels were particularly higher in the cultured peritoneal macrophages than in the serum, but were not statistically different between the 2 groups.

Conclusion: Our results showed that the beneficial

immune response obtained in the CO₂ gas insufflation laparoscopic procedure could also be obtained in the gasless abdominal wall lift laparoscopic procedure. An immediate preservation of the immune functions in the postoperative period was detected similarly in the 2 groups.

Key Words: Immune preservation, Laparoscopic cholecystectomy, Carbon dioxide insufflation, Abdominal wall lift, Gasless.

INTRODUCTION

Ever since Mouret¹ of France first performed a laparoscopic cholecystectomy in 1987, and Reddick and Olsen in the United States reported on it, laparoscopic surgery has spread rapidly throughout the world. Laparoscopic surgery is currently used actively in a variety of surgical conditions. Many physiological changes of laparoscopic surgery have recently been reported in some basic research.²⁻⁴ Among these studies, it has already been established as a theory that the conventional laparoscopic surgery under CO₂ gas is immunologically superior to laparotomy.⁵ Substantial experimental and clinical evidence also exists to indicate that the immune response is better preserved after laparoscopic surgery,⁵⁻¹¹ but the mechanism for immune preservation under the CO₂ gas-specific effect is still unclear in all situations.

To elucidate the immunologic aspects of laparoscopic surgery, we measured and compared clinical patients who underwent cholecystectomy with the gas (CO₂) technique and the gasless abdominal wall lift technique by observing the immune responses of the 2 groups through measuring various immunologic factors.

MATERIALS AND METHODS

Patients and Procedures

The study was conducted with 50 patients who were diagnosed with cholelithiasis at Catholic University Hospital, Seoul, Korea, between March 1998 and February 1999. The patients were randomly divided into

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a gas laparoscopic cholecystectomy group and an abdominal wall lift laparoscopic cholecystectomy group, each group with 25 patients. Those patients whose cholelithiasis was accompanied by cholecystitis or other complications were excluded from the study. Those patients who were on medication and those with a past history of diseases that could cause a reduced immune function were also excluded.

An informed consent was obtained from all of the patients in this study, and the study was conducted following the guidelines of The Catholic Central Hospital Clinical Research Management and of the Declaration of Helsinki. The patients did not know which technique would be used before the operation, and the technique was selected randomly before anesthesia.

The same medications were used for anesthesia in both groups. The surgical technique used in the gas laparoscopic cholecystectomy group included maintaining the CO₂ pressure at 12 mm Hg. Laparoscopic cholecystectomy was performed using 4 trocars, two 10-mm trocars and two 5-mm trocars. In the wall lift laparoscopic cholecystectomy group, the basic principle was to use a Kim's lifter (Sejong Medical, Paju, Korea) with a cushion and membrane retractor (Sejong Medical, Paju, Korea), and the same 4-trocar method was used as in the gas laparoscopic cholecystectomy. None of the patients had a history of transfusion. If possible, we tried to match up the age, weight, and surgery time between the 2 groups (**Table 1**). Also, to compare the presence of infection and hormonal conditions before and after the surgery, the erythrocyte sedimentation rate (ESR), white blood cell (WBC) count and cortisol levels were measured and com-

pared at preoperative 24 hours, and at postoperative 24 and 72 hours. The time of surgery was expressed in minutes from the time of incising to suturing the skin.

Measurement of Immunologic Parameters in the Peripheral Blood

The immunologic parameters in the peripheral blood were obtained by measuring the lymphocyte level at preoperative 24 hours, and postoperative 24 and 72 hours. We also obtained an extra 10 cc of blood to measure the levels of certain cytokines (TNF- α , IFN- γ , IL-6, and IL-8) by centrifuging the blood at 2000 rpm for 20 minutes and using ELISA (Cytoscreen immunoassay kit, Biosource International Co., CA, USA).

Estimation of the Production of Peritoneal Macrophage-Derived Cytokines

The production of the peritoneal macrophage-derived cytokines was estimated with 1 liter of the normal saline solution that had been used to irrigate around the peritoneum immediately after the cholecystectomy in the patients of both groups. Six mL of prechilled PBS (pH 7.2) was added to this irrigated saline solution, and the mixture was centrifuged for 5 minutes. The mixture was resuspended in RPMI-1640 medium (Gibco BRL Co., NY, USA) containing 10% fetal bovine serum (FBS, Gibco BRL Co., NY, USA). The mixture was divided into a 7.5 x 10⁵/1-mL well so that the viability of the macrophages would be more than 95%. The mixture containing the cells was then divided into tissue culture multiple-well plates where it was preincubated for 1 hour. The supernatant was discarded, and the plates were washed 2 times with 1 mL of sterile PBS. After adding 1 mL of fresh

Table 1.
Patient Characteristics.

	Gas Group (n = 25)	Wall Lift Group (n = 25)
Mean (\pm SD)†age, yr	46 \pm 12	44 \pm 13
Sex ratio (male/female)	5 : 20	5 : 20
Mean (\pm SD) weight, kg	63 \pm 6	63 \pm 7
Mean (\pm SD) operative time min	51 \pm 20	61 \pm 11

*Statistically not significant in the 2 groups

†Standard deviation

medium, the plates were cultured for 4 hours, and the supernatant was obtained and kept at 20°C. The TNF- α , IFN- γ , IL-6, and IL-8 levels were measured with the method described previously.

Statistical Analysis

All the measurements were expressed as the average \pm standard deviation, and the 2 groups were compared and analyzed for age, weight, and the time of surgery with the unpaired *t* test. The comparison of the immunologic measurement factors in the blood between the 2 groups was evaluated with ANOVA for repeated measurements, and the unpaired *t* test was also used for the other measurements between the 2 groups (such as the comparisons at each time period and immunologic measurement factors within the peritoneum).

RESULTS

Table 2 provides a comparison of data between CO₂ insufflation group and abdominal wall lift group.

Hormonal and Inflammatory Markers

No statistical difference existed between the blood cortisol levels or the time periods of the 2 groups (*P* > 0.10). Neither was there a statistical difference between the ESR (*P* > 0.46) and white blood cell count (*P* > 0.95).

Blood Cytokines

No statistical differences existed between the CO₂ gas and wall lift laparoscopic cholecystectomy groups in terms of the measured levels of TNF- α (*P* > 0.45), IFN- γ (*P* > 0.71), IL-6 (*P* > 0.48), and IL-8 (*P* > 0.72).

Blood Cytokine Levels at Each Time Period

The changes in the levels of the cytokines at 24 hours and 72 hours postoperative decreased significantly in both the CO₂ gas and wall lift laparoscopic cholecystectomy groups (*P* < 0.0001).

Values of Peritoneal Macrophage-Derived Cytokines Production

The cytokine levels were significantly higher in the cultured peritoneal macrophages than in the serum, but no statistical differences existed between the CO₂ gas and wall lift laparoscopic cholecystectomy groups with respect to the measured levels of TNF- α (*P* > 0.45), IFN- γ (*P* > 0.89), IL-6 (*P* > 0.35), and IL-8 (*P* > 0.73).

DISCUSSION

Minimally invasive surgery has gained broad acceptance, and its use has increased tremendously in recent years. The basic science research about laparoscopic surgery has contributed to this trend. Many authors have demonstrated that laparoscopic surgery preserves immune function, so the immunologic advantages of laparoscopic surgery have become well known.

Several mechanisms have been used to explain the superiority of laparoscopic surgery over laparotomy from an immunologic aspect. The most widely held principle is that of the CO₂ response in intraperitoneal immunity. Carbon dioxide forms carbonic acid in an aqueous environment, and a drop in pH after the induction of CO₂ pneumoperitoneum could affect the biochemical and cellular immune function inherent to the peritoneal cavity.⁴ The mechanism of whether CO₂ gas itself affects immune function positively, as described previously, is convincing; but it is still open to discussion. Therefore, to detect the real CO₂ gas effect, we checked for several appropriate immunologic factors that can measure immune function and compared the differences between the gas and abdominal wall lift techniques.

In this study, intraperitoneal immunity and peritoneal response were investigated with respect to the release of peritoneal cytokines. The cytokine levels were checked in the peripheral blood at 24 hours and 72 hours after the operation to detect the consecutive postoperative immune response of the 2 groups. As for the immunologic parameters, 4 kinds of cytokines were checked, of which tumor necrosis factor- α is known to be related to the secondary injury-related neuroendocrine response to damage after trauma or surgical injury. This factor is activated by INF- γ and is secreted from macrophages.^{11,12}

Interferon- γ activates certain cells, such as macrophages, is known to increase the oxidative and cytotoxic functions of the macrophages, and is the cytokine that increases after surgical damage.¹¹ Interleukin-6 promotes the differentiation of the B-lymphocytes as well as the production of immunoglobulin, it affects the acute phase reactant and production of prostaglandin,¹³ and it induces the chemotaxis response of the lymphocytes and macrophages at the damage site. Interleukin-8 has recently drawn attention as the index of the chemotaxis response of neutrophils, which are the basic component of acute inflammatory response.¹¹ Accordingly, measuring the changes in the immunologic responses that include

Table 2.
Results of the Prospective Study.*; †

	CO ₂ insufflation	Wall Lift
ESR (erythrocyte sediment rate) (mm/hr)		
preoperative	6.88 ± 2.89	6.36 ± 2.27
1 POD	35.36 ± 9.03	37.04 ± 7.28
3 POD	29.04 ± 8.38	31.40 ± 10.28
Cortisol (µg/dL)		
preoperative	10.05 ± 2.83	9.65 ± 2.25
1 POD	15.32 ± 2.99	14.0 ± 2.90
3 POD	18.68 ± 3.42	17.07 ± 4.0
White blood cells (/uL)		
preoperative	5552 ± 1157	5500 ± 1017
1 POD	8328 ± 2105	7932 ± 1850
3 POD	5872 ± 1383	6264 ± 2055
TNF-α (serum) (pg/mL)		
preoperative	6.86 ± 15.0	5.61 ± 9.94
1 POD	510 ± 244.52	540 ± 224.53
3 POD	90 ± 31.66	115 ± 73.68
INF-γ (serum) (pg/mL)		
preoperative	5.62 ± 14.17	6.86 ± 15.66
1 POD	510 ± 274.62	530 ± 263.39
3 POD	150 ± 74.38	157.5 ± 80.92
IL-6 (serum) (pg/mL)		
preoperative	1.24 ± 4.31	1.87 ± 6.85
1 POD	365 ± 148.42	390 ± 12.05
3 POD	90 ± 31.66	95 ± 31.86
IL-8 (serum) (pg/mL)		
preoperative	2.24 ± 3.33	1.12 ± 2.61
1 POD	464.40 ± 258.37	484.80 ± 260.04
3 POD	86.40 ± 32.0	92.8 ± 44.30
TNF-α(cultured peritoneal macrophages) (ng/mL)	900 ± 204.12	940 ± 165.83
INF-γ(cultured peritoneal macrophages) (ng/mL)	710 ± 266.92	720 ± 253.31
IL-6(cultured peritoneal macrophages) (ng/mL)	450 ± 102.06	475 ± 88.38
IL-8(cultured peritoneal macrophages) (ng/mL)	627.60 ± 222.30	648 ± 208.20

*Statistically not significant between the two groups (Mean ± standard deviation)

†Statistically significantly decreased the serum cytokine levels between 1 POD and 3 POD (*P* < 0.0001)

these factors, the cellular and humoral immunologic mechanisms, and other immune responses could be measured accurately, either directly or indirectly, and used as the indices of immune response in the body.

The current investigation suggests that systemic and local intraperitoneal immunity appear to be better preserved following CO₂ pneumoperitoneal laparoscopic interventions.⁴ However, in spite of a slightly increased cytokine level in the wall lift group, this study showed no statistical immunologic differences in the 2 groups. The immunity was evenly preserved immediately after surgery in each group. With these results, we could not explain the suggestion that a CO₂ gas-specific effect can induce immunological advantages in laparoscopic surgery. To detect a more accurate mechanism for the immunologic effect with laparoscopic surgery, all other possible factors must be analyzed through clinical research. In this study, plasma cortisol levels and the ESR and WBC counts were also assessed as markers of stress hormone activity and acute inflammatory response. It is interesting to note that no differences were observed in this study group.

Our findings suggest that the minimally invasive techniques (carbon dioxide insufflation and the gasless technique) are both able to preserve early postoperative immunity. It can therefore be concluded that the immune preservation of laparoscopic surgery is not the response to the CO₂ gas itself, but can be explained by changes due to other factors. Furthermore, it was determined that the superior preservation of immunity that is achieved with conventional laparoscopic surgery can also be obtained through the gasless abdominal wall lift technique. As demonstrated by cytokine production during the early and middle phases of the postoperative period in both scenarios, further research is needed to determine whether the CO₂ gas is associated with an immunologic advantage in minimally invasive surgery, and further research is needed to clarify the specific immune mechanism in minimally invasive surgery.

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