

Research Article

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Serum inflammatory cytokines comparison in gastric cancer therapy

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Abstract: To compare serum inflammatory cytokines between laparoscopic-assisted and open radical gastrectomy in the perioperative period, 80 cases of advanced gastric cancer were chosen for the study. They were divided into laparoscopy group (40 cases) and abdominal open surgery group (40 cases), performed laparoscopic-assisted radical gastrectomy and conventional open radical gastrectomy, respectively. Serum Heme oxygenase-1 (HO-1), TNF- α , IL-6 and CRP were measured by ELISA on preoperative day 1, post-operative day 1 and post-operative day 3. Serum HO-1, TNF- α , IL-6 and CRP had no significant difference between the laparoscopy group and the open group on pre-operative day 1. Serum HO-1, IL-6 and CRP of the laparoscopy group were significantly lower than that of the open group on post-operative day 1 and day 3 except for Serum TNF- α which had no significant difference. Laparoscopic-assisted radical gastrectomy was minimally invasive compared with conventional open radical gastrectomy in advanced gastric cancer patients.

Keywords: Gastric carcinoma; Laparoscope; Heme oxygenase-1; Tumor necrosis factor- α ; Interleukin-6

1 Introduction

Gastric cancer is one of the most severe cancers in the world. It has led to many deaths every year [1]. Currently, the major methods for gastric cancer therapy are Surgery, chemo-therapy and radiotherapy. There are also some other methods in clinical or basic research on gastric cancer therapy [2-6]. In surgery, laparoscopy had been

widely used for early gastric cancer therapy. While controversy still existed for employing laparoscopic-assisted radical gastrectomy on progressive gastric cancer patients [7]. Researchers found that laparoscopic-assisted radical gastrectomy on progressive gastric cancer patients had many advantages such as a speedy postoperative recovery, short hospitalized time, while the safety, extent of curative and long-term survival were equivalent to open surgery [8].

In the year of 1994, Kitano et al performed the first case of laparoscopic radical gastrectomy for gastric cancer and the application of laparoscopic technique in early gastric cancer had been promoted rapidly from then on [9]. In 2004, Japanese Society of gastric cancer issued Guidelines for the treatment of gastric cancer which had designed laparoscopic operation as one of the standard therapeutic method for early gastric cancer. As the development of instrument and promotion of operational technique, laparoscopic radical gastrectomy for gastric cancer had an extension in advanced gastric cancer patients. Laparoscopic operation had smaller incision and more clear vision compared with abdominal open surgical. The range of tumor resection and number of lymph nodes cleared between the two operation methods had no significant difference. But when employed laparoscopic operation in advanced gastric cancer patients, long time pneumoperitoneum would bring abdominal hypertension syndrome, hypercapnia caused by CO₂ entering into blood and ischemia reperfusion injury caused by abdominal pressure drop while operation finished [10]. So the laparoscope used in the advanced gastric cancer still had controversy.

IL-6 is an important cytokines that takes part in the systemic stress response and immunoregulation. IL-6 mainly comes from T cells, B cells and mononuclear macrophage and is the major participant of tissue damage and stress response. The level of it will significantly elevate while in operation, trauma or stress.

CRP is a kind of acute phase reactive protein, which is a sensitive indicator of the order of severity of tissue damage. It is synthesized by hepatocytes under the influ-

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ence of IL-6. The level of CRP in the peripheral blood will increase while the body is in a state of stress.

TNF- α generates from active mononuclear macrophages and is vitally important for immune function [11]. It also induces inflammatory chain reactions, thus promoting the release of IL-1, IL-6, IL-8, platelet activating factor, prostaglandin, leukotriene and leukotriene D4 or C4 which could cause a general reaction, resulting in the activation of inflammatory effective cells such as mononuclear macrophages, granulocytes and lymphocytes, coagulation systems and complement systems which caused injure of vascular endothelial cells and step further induced tissue and organ damage. Trauma of abdominal surgery especially peritoneum surgery could increase macrophage activity of the entire body, especially the abdominal cavity, resulting in the secretion of inflammatory factors such as TNF- α and IL-6.

HO-1 could catalyze heme to biliverdin, CO and iron ion and is vitally important for hem metastasis. HO-1, heat shock protein 32, is a kind of stress protein which could be induced by many factors such as trauma, surgery, oxidative stress, ischemia, anoxia, cytokines, heme, endotoxin and heavy metals [12, 13] to anti-inflammatory [14], anti-apoptosis [15], protect cells and protect organ transplanted [13, 16-19].

To certify whether laparoscope should be used in advanced gastric cancer therapy or not, in this study, 80 cases of advanced gastric cancer patients were chosen and divided into the laparoscopic-assisted group (40 cases) and the abdominal open group (40 cases) randomly. Then the serum samples were collected and concentrations of serum HO-1, TNF- α , IL-6 and CRP were detected by ELISA method which aimed to observe the change rules of inflammatory along the time and compare the level differences of inflammatory between the two groups, to further evaluate the viability of laparoscopic-assisted radical gastrectomy on advanced gastric cancer patients.

2 Material and method

2.1 General material

80 cases of advanced gastric cancer patients were chosen, who were employed radical operation at Gastrointestinal Surgery of Shandong university affiliated Provincial Hospital in time of April to September, 2014. They were divided into the laparoscopy group (40 cases) and the abdominal open surgery group (40 cases) (see table 1). Operation of the two groups' of patients was performed by doctors of

the same surgery group. All of the patients were examined by laparoscopy and diagnosed with gastric cancer by biopsy pathology. Auxiliary examination before operation excluded widespread metastasis in the abdominal cavity and distant metastasis in other organs. All of them had no heavy heart, lung, liver, brain, kidney and immune system syndromes, no abdominal operation history and had not received radical or chemical therapy before operation. The patients with the following characters were excluded from this study, such as the patients with pneumoperitoneum contraindications, patients applied immune enhancers or inhibitors before, in or after operation, patients injected blood products in the perioperative period, patients who were found with tumor widely metastasis that invaded the adjacent organ or blood vessels then changed to employ extended radical cure or palliative resection or switch operation and the patients who were hard to do laparoscopic operation or other reasons to employ open abdomen surgery.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

Informed consent: Informed consent has been obtained from all individuals included in this study.

2.2 Anesthesia and surgery

Both of the two groups were employed intravenous and tracheal intubation anesthesia. All the patients in the laparoscopy group employed laparoscopy assisted radical gastric cancer operation. 28 of the cases had laparoscopic distal gastrectomy, 9 cases had proximal gastrectomy and 3 of them had total gastrectomy. All the incisions of the abdominal wall were 5-6 centimeters located at the upper abdominal midline subxiphoid. The pneumoperitoneum pressure during operation maintained at 12-15 mmHg. The patients of the open group employed traditional open radical gastrectomy for gastric cancer. 30 of the patients had distal gastrectomy, 8 cases had proximal gastrectomy and 2 cases had total gastrectomy. The resection ranges in the open group were equivalent to the laparoscope group. All lymph node dissection of the patients were according to the 13th edition of gastric cancer treatment protocol of gastric cancer staging method employing D2 radical resec-

tion, eliminating the first and second stations of lymph nodes in the stomach.

2.3 Observed indicators

Extract the fasting peripheral venous blood 2ml on preoperational day 1 and post-operation day 1 and day 3 separately, centrifuge for 20min in 2500r/min, then store the supernatant in -80°C waiting for test after sample collection. Examine the concentration of serum HO-1, TNF- α , IL-6 and CRP by ELISA. (HO-1 kit bought from Enzo Lifesciences Company, TNF- α , IL-6 and CRP kit bought from RD company)

The minimum detectable dose was 0.106ng/L for TNF- α , 0.70ng/L for IL-6, 10ng/L for CRP and 49ng/L for HO-1. The assays were performed directly according to the instructions of the manufacturer. The serum for TNF- α and IL-6 detection weren't diluted, while the pre-operation day1 samples for CRP detection were diluted 100 folds and diluted 4000 folds for post-operation day1 and day3 samples. All the samples for HO-1 detection were diluted 16 folds.

2.4 Statistical method

SPSS software was used here and the data showed as data \pm standard ($\bar{C} \pm s$). Paired T-test and group data T-test were used for mean comparison of different groups. $P < 0.05$ stands for significant difference.

3 Results

3.1 General data comparison between two group patients

Two patient groups had a good recovery post-operation and had no complications. The gender, age, body mass index, site of resection and number of cleared lymph nodes of the patients had no significant difference. The operation time of the laparoscope group was significantly longer than the open group. The amount of bleeding in the operation and hospital days of the laparoscope group was significantly lower than the open group (Table 1).

3.2 Level difference of serum TNF- α , HO-1, IL-6 and CRP pre- and post-operation in LG and OG

The levels of serum HO-1, TNF- α , IL-6 and CRP in day1 and day3 post-operation of the two groups were significantly higher than pre-operation ($P < 0.05$). Compared between the two groups, the levels of serum HO-1, TNF- α , IL-6 and CRP pre-operation had no difference and the levels of HO-1, IL-6 and CRP in day 1 and day 3 post-operation in laparoscope group were significantly lower than open group, while the level of TNF- α in day 1 and day 3 post-operation hadn't significant difference. (Figure 1 and Table 2)

3.3 Discussion

It is consensus that the increase of IL-6 and CRP is proportion to degree of surgical trauma [20]. Research here showed that the levels of serum IL-6 and CRP in day 1 and day 3 after operation were significantly higher than before

Table 1: General data comparison between two group patients

Item	Laparoscope group	open group	P
Gender (male/female, case)	25/15	23/17	0.820
Age (year)	55.58 \pm 10.15	52.65 \pm 6.62	0.131
Weight (kg/m ²)	20.65 \pm 2.04	21.03 \pm 2.37	0.444
Location(far/near/total gastric, case)	28/9/3	30/8/2	0.849
Surgical duration (min)	245.18 \pm 42.75	184.27 \pm 34.72	<0.001
Amount of bleeding (ml)	78.40 \pm 40.69	98.31 \pm 48.05	0.049
Number of lymph nodes cleaned	30.13 \pm 9.58	31.42 \pm 11.47	0.587
Hospital stays (day)	9.26 \pm 1.75	11.31 \pm 2.08	<0.001

Table 2: Comparison of indexes in different times between two group patients

Group	Laparoscope group	Open group	P1	R (P2)
HO-1 (ug/L)				
Pre-operation day1	8.54±3.38	9.64±4.59	0.227	
Post-operation day1	14.70±8.59*	20.81±9.28*	0.005	
Post-operation day3	22.72±9.70*	31.59±10.57*	<0.001	
TNF-α(ng/L)				
Pre-operation day1	1.70±0.51	1.85±0.45	0.140	
Post-operation day1	2.18±0.50*	2.25±0.55*	0.584	0.584
Post-operation day3	2.61±0.73*	2.61±0.52*	0.968	
IL-6(ng/L)				
Pre-operation day1	1.62±0.93	1.17±1.53	0.121	
Post-operation day1	83.36±28.52*	103.43±41.29*	0.013	0.148(0.368)
Post-operation day3	14.15±5.95*	12.55±4.26*	0.170	
CRP(mg/L)				
Pre-operation day1	3.04±1.59	2.84±1.52	0.572	
Post-operation day1	80.39±10.95*	94.06±20.27*	<0.001	0.548(<0.001)
Post-operation day3	95.73±21.66*	104.95±21.49*	0.065	

Note: Compared with pre-operation, * stands for $P < 0.05$.

P_1 stands for comparison of the same time between two groups.

R stands for the correlation of other three inflammatory factors with HO-1.

operation for both the laparoscope group and open surgical group. Compared between the two groups, the levels of IL-6 and CRP before operation both had no obvious difference, but in day1 and day 3 after operation, the serum IL-6 and CRP of the laparoscope group were significantly lower than the open surgical group. These results showed that the trauma of the laparoscopic radical gastrectomy was less than the open gastric cancer radical prostatectomy which coincided with the study result of SsangYong Li et al on colon cancer [19].

Another papers reported that IL-6 could activate hypothalamus-pituitary-adrenocortical axis and increase the level of adrenocortical hormone which had immunosuppressive functions [21]. This research here showed

that the level of serum IL-6 after operation in the laparoscope group was lower than the open group thus the laparoscopic radical gastrectomy had a weaker immunosuppressive function than the open gastric cancer radical prostatectomy [17]. Researchers also found that compared with laparotomy, surgically assisted by laparoscope had a weaker immunosuppressive effect post-operatively [18]. Cancer relapse was closely related with systemic immune function, so laparoscopic radical gastrectomy probably reduced the risk of tumor recurrence.

The study of Ordemann et al found that the level of serum TNF-α after colorectal operation was significantly higher than before operation in both the laparoscope group and the open surgery group, while the peak of

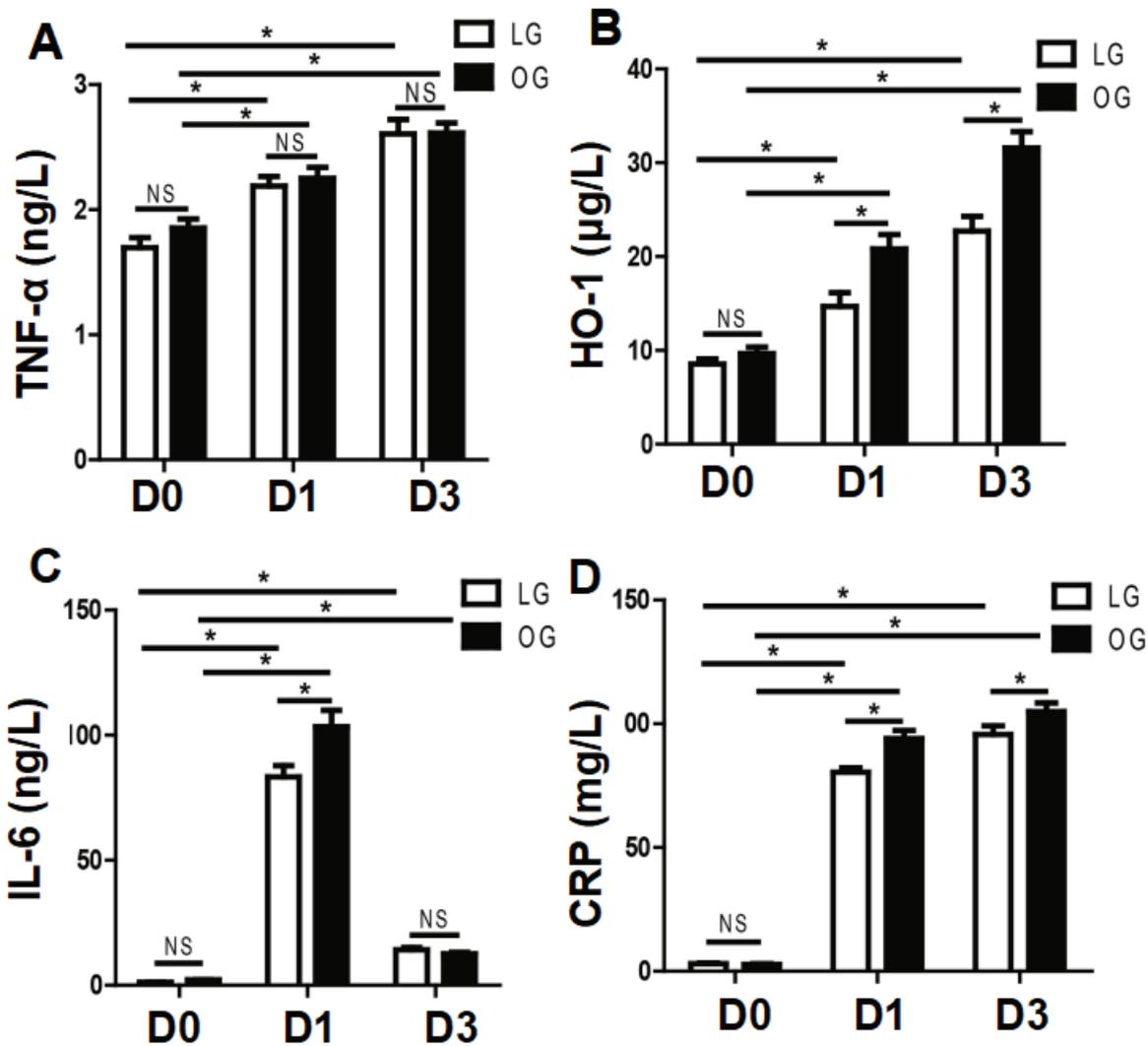


Figure 1: Level difference of serum TNF- α , HO-1, IL-6 and CRP pre- and post-operation in laparoscope group (LG) and open surgical group (OG). A. serum TNF- α difference pre- and post-operation in LG and OG; B. serum HO-1 difference pre- and post-operation in LG and OG; C. serum IL-6 difference pre- and post-operation in LG and OG; D. serum CRP difference pre- and post-operation in LG and OG. * stands for $P < 0.05$. NS stands for no significant difference ($P > 0.05$).

TNF- α in the laparoscope group was significantly lower than the open surgical group [22]. Our research found that in advanced gastric cancer patients, the serum TNF- α after operation was significantly higher than before operation for both the laparoscope group and open group, but no significant difference existed between the two groups before or after operation.

Study showed that HO-1 level could be an inflammatory indicator. Our research showed that HO-1 level in day 1 and day 3 after operation was significantly higher than before operation for both of the two groups. Compared between the two groups, HO-1 level before operation had no significant difference, but the HO-1 level in day 1 and day 3 after operation of laparoscope group was significantly

lower than the open surgical group which showed that the laparoscope group caused a weaker inflammatory reaction and had lighter damage.

In recent years, relationships between HO-1 and the other inflammatory factors became hot research. Studies showed that HO-1 could be positively regulated by IL-1 β , TNF- α and LPS [23], while increase of HO-1 could inhibit the expression of IL-1 β , IL-6, TNF- α and MMP-9 by anti-inflammatory [18, 24]. Thus HO-1 had a negative regulation for inflammatory factors to prevent infinite amplification of inflammatory reaction. This research showed that serum HO-1 positively correlated with TNF- α and CRP, so we proposed that an increase of HO-1 after operation was caused by trauma directly and also probably be caused by

increased stimulation of inflammatory factors at the same time. We had having a follow-up for all the patients, but it was meaningless still for short time. Due to the simple design of this experiment and the lack case number, the relationships between HO-1 and TNF- α , CRP, IL-6 remains to be further research. In the treatment options to gastric cancer, nanomaterials and hydrogels may be good ways to develop therapeutic approaches [25-26].

Above all, in advanced gastric cancer, concentrations of serum HO-1, IL-6 and CRP after operation of laparoscopic assisted operation were lower than traditional open laparotomy which verified the advantage of laparoscopic minimally invasive operation. Compared with open laparotomy, lower inflammatory level of laparoscopic radical gastrectomy had a weaker immunosuppressive effect on the body thus probably leading to a better prognosis. HO-1 as a protective protein, the interactive functions with other inflammatory factors remain to be further explored. Long-term prognosis of laparoscopic assisted radical therapy in the perioperative period of advanced gastric cancer will be studied further.

4 Conclusion

In summary, Serum HO-1, TNF- α , IL-6 and CRP had no significant difference between the laparoscopy group and the open group on pre-operative day 1. Serum HO-1, IL-6 and CRP of laparoscopy group were significantly lower than that of the open group on post-operative day 1 and day 3 except for Serum TNF- α . Laparoscopic-assisted radical gastrectomy was minimally invasive compared with conventional open radical gastrectomy in advanced gastric cancer patients.

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Conflicts of Interest: The authors declare no conflicts of interest.

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