

Research paper

The growth endocrine axis and inflammatory responses after laparoscopic cholecystectomy

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ABSTRACT

OBJECTIVE: It is well known that conventional surgery leads to detrimental immune and catabolic responses, thus there is growing interest in the effect of minimally invasive techniques on postoperative endocrine and immune function. The aim of this prospective study was to evaluate the growth hormone (GH)/insulin-like growth factor-1 (IGF-1)/IGF binding protein-3 (IGFBP-3) axis and acute phase (interleukin-6, IL-6, and C-reactive protein, CRP) responses in patients who underwent laparoscopic cholecystectomy. **DESIGN:** Twenty-nine patients (16 women, 13 men; age: 58±8 years) with a history of uncomplicated symptomatic cholelithiasis participated in the study. Blood samples were collected prior to and at 24 hrs and 48 hrs after laparoscopic cholecystectomy. Serum concentrations of GH, IGF-1, IGFBP-3, and IL-6 were determined by standard sandwich enzyme-linked immunosorbent assay (ELISA), while CRP was measured by nephelometry. ANOVA with repeated measures and Tukey's post-hoc test were used to evaluate changes in serum measurements. **RESULTS:** The laparoscopic cholecystectomy resulted in a significant postoperative increase in circulating levels of IL-6 (p=0.031), which is the main cytokine responsible for inducing the acute inflammatory response, and of the acute phase protein CRP (p=0.005). A significant increase in GH levels at 24 hrs (p=0.034) and decrease of IGF-1 on both postoperative days were also found (p=0.045, 0.044), while no changes were documented in IGFBP-3 levels. Significant correlations were revealed between postoperative levels of the acute phase proteins and growth axis hormones (p<0.05 - 0.001). **CONCLUSIONS:** Our findings suggest that laparoscopic cholecystectomy induces acute phase endocrine and immune responses. These changes may represent a state of systemic inflammation and GH resistance, compatible with possible cytokine-induced anti-anabolic or catabolic effects even after this minimally invasive cholecystectomy.

Key words: Acute phase response, Growth hormone, Inflammation, Insulin-like growth factor-1, Interleukin-6, Laparoscopy

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INTRODUCTION

Over the last decade, an increasing number of studies have been conducted to investigate the effects of minimally invasive surgical techniques on surgical stress response and a variety of immune function parameters.¹⁻⁴ The introduction of advanced laparoscopic methods has revealed several clinical advantages of minimally invasive surgery,^{1,3,5} including less postoperative pain, quicker recovery, and shorter hospital stay.^{2,6-8}

Nevertheless, although laparoscopic techniques are being increasingly used in the management of various intra-abdominal conditions, there is conflicting evidence regarding the effect of laparoscopic surgery on postoperative systemic inflammatory response. Specifically, concern has been raised that increased intra-abdominal pressure during laparoscopy may promote bacteremia and systemic inflammation,^{5,9} while recent findings suggest that carbon dioxide pneumoperitoneum could have a protective effect against postoperative enhanced systemic inflammation, particularly after laparoscopic cholecystectomy.^{3,10,11}

Immunity and inflammation after surgery are mediated by cytokines, such as tumour necrosis factor- α (TNF- α), interleukin (IL)-1, and IL-6, which are activated as an early response to tissue injury.¹²⁻¹⁴ Specifically, IL-6 is the main cytokine responsible for inducing the systemic changes known as the acute phase response, which includes the production of acute phase proteins in the liver, such as C-reactive protein (CRP), and plays a major role in inflammation.¹⁴⁻¹⁷

Moreover, surgical stress response is characterized by increased secretion of pituitary hormones, decreased secretion or effects of anabolic hormones, and hypermetabolism. The overall metabolic effect of the tissue injury-induced endocrine and metabolic changes is increased catabolism, and those changes are thought to mediate the increased demand on the reserves and immune competence of patients post surgery.^{2,14,18} In particular, growth hormone (GH) is secreted by the anterior pituitary and its secretion increases in relation to the severity of tissue injury after surgery.¹⁴ Many GH actions are mediated by insulin-like growth factor-1 (IGF-1), which, in the context of its endocrine activity, is produced and secreted by the liver, skeletal muscle, and other tissues

in response to stimulation by GH.¹⁹⁻²¹ Furthermore, it has been proposed that acute inflammation induces a GH-resistant state as part of a regulatory mechanism of the body to restrict growth and energy storage, in which the elevated GH secretion is not followed by increased circulating IGF-1 levels.²²⁻²⁴

Although the effect of surgical stress and/or inflammatory responses on endocrine function after laparoscopic surgery has been described in humans,^{2,13,25-27} studies designed to detect the relation between the response of the anabolic GH/IGF-1 axis and laparoscopic cholecystectomy in the context of an acute phase response are lacking. This prospective study aimed at determining whether there are acute phase changes in growth axis hormones in patients who underwent laparoscopic cholecystectomy.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were in accordance with the ethical standards of the Ethics Committee of the Institutional Review Board of "Laiko" General Hospital, which approved the study, and with the Declaration of Helsinki. Written informed consent was provided by all volunteers participating in this study.

Patients

From September 2013 to May 2014, a hospital-based prospective study was conducted in 29 consecutive patients (16 women, 13 men; age 58 \pm 8 years). They had a history of uncomplicated symptomatic cholelithiasis and were referred to the authors' department for surgical treatment of their disease. The patients were scheduled for laparoscopic cholecystectomy and had not received any treatment for at least a week before they underwent the surgical excision of the gallbladder. In all patients, general anesthesia was induced with propofol or midazolam, while postoperative analgesia consisted of routine administration of fentanyl or paracetamol. The choice of anesthetics was determined by the attending anesthesiologists. Patients were released from hospital on the third postoperative day.

Blood sampling and serum measurements

Blood samples were collected between 8:00 and 9:00 am from each individual patient before the op-

eration (during the patients' preoperative medical examination) and after the laparoscopic removal of the gallbladder (cholecystectomy), at 24 and 48 hrs. The time points of blood sampling were chosen to cover the period of acute phase response following laparoscopic surgery. The patients were at rest for at least 30 min prior to collection of 10 mL of blood obtained from an antecubital vein. Blood samples were allowed to clot at room temperature for 30 min. Serum was collected following centrifugation at 2000 g at 4°C for 10 min, stored frozen in 0.2 mL aliquots at -80°C, and thawed once only at the time of analysis. CRP was measured by latex-enhanced nephelometry (Dade Behring Nephelometer II Analyzer System, BNII, Siemens, Erlangen, Germany). Serum concentrations of IGF-1, insulin-like growth factor binding protein (IGFBP)-3, GH, and the acute phase protein IL-6 were determined by standard sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available kits (IGF-1: Enzo Life Sciences, Michigan, USA; IGFBP-3 and GH: R&D Systems Inc., Minneapolis, USA; IL-6: Biologend Inc., San Diego, USA) according to the manufacturer's instructions. Briefly, 96-well microtiter plates (Costar) were coated with a monoclonal antibody directed against the analyte. Samples and standards were applied and the bound analyte was detected with horseradish peroxidase conjugated to a secondary polyclonal antibody directed against the analyte. Visualization of the presence of the peroxidase label was achieved with a tetramethylbenzidine (TMB) substrate. Colour formation was measured with a microplate reader (Versamax, Molecular Devices, Sunnyvale, CA, USA) at 450 or 540 nm, and calculations were performed using SoftMax Pro software (Molecular Devices, Sunnyvale, CA, USA). All samples were analyzed simultaneously, in duplicate and the results averaged. According to the manufacturers, the minimal detection limits of the assays were 34.2 pg mL⁻¹, 50 pg mL⁻¹, 2.1 pg mL⁻¹, 1.6 pg mL⁻¹ for IGF-1, IGFBP-3, GH and IL-6, respectively. The intra- and inter-assay coefficients of variation (CV) were as follows: 3.6%-8.9% and 3.4%-10.9% for IGF-1, 2.3%-5.0% and 5.4%-8% for IGFBP-3, 2.4%-4.1% and 6.9%-9.4% for GH, and 4.3%-11.3% and 4.5%-13% for IL-6.

Statistical analysis

A one-way analysis of variance (ANOVA) with

repeated measures over time was employed to evaluate changes in all serum measurements (SPSS v. 21 statistical package; SPSS Inc., Chicago, IL, USA). Where significant F ratios were found for main effects ($p < 0.05$), the postoperative mean values were compared with the preoperative means (control) using Dunnett's post hoc tests. Relationships between variables were examined using Pearson's correlation coefficient (r). All data are presented as mean \pm SE. Statistically significant changes were considered at $p < 0.05$.

RESULTS

The coefficient r^2 for standard curves of all the ELISA analyses was 0.994-1. Analysis of the inflammation-related factor IL-6 in the blood revealed that the laparoscopic cholecystectomy resulted in a significant three-fold increase of circulating IL-6 levels on postoperative day 2 (48 hrs) compared to the preoperative (PRE) levels (17.9 \pm 4.6 vs. 5.2 \pm 1.9 pg/ml; F ratio: 3.142, $p=0.045$, Table 1). Similarly, there was a significant five-fold increase in the serum levels of the acute phase protein CRP on postoperative day 2 compared to the preoperative levels (20.9 \pm 5.6 vs. 3.87 \pm 0.28 mg/L; F ratio: 4.965, $p=0.010$, Table 1). A significant two-fold increase was observed in the levels of GH on postoperative day 1 (24 hrs) before returning to the preoperative levels on day 2 (PRE levels: 520.6 \pm 141.2 vs. 1100.9 \pm 216.1 vs.

Table 1. Serum concentrations of the growth axis hormones GH, IGF-1, and IGFBP-3, and of the acute phase proteins IL-6 and CRP, preoperatively (PRE) and on days 1 and 2 post laparoscopic cholecystectomy. P values refer to comparisons with the preoperative concentration (mean \pm SE)

	PRE	Day 1	Day 2
GH (pg/ml)	520.6 \pm 141.2	1100.9 \pm 216.1 (p=0.034)	505.6 \pm 142.8 (p=0.997)
IGF-1 (ng/ml)	130.9 \pm 12.3	110.6 \pm 13.2 (p=0.045)	110.5 \pm 11.1 (p=0.044)
IGFBP-3 (μ g/ml)	1.95 \pm 0.14	1.96 \pm 0.13 (p=1.000)	1.96 \pm 0.14 (p=0.997)
IL-6 (pg/ml)	5.2 \pm 1.9	11.9 \pm 3.0 (p=0.319)	17.9 \pm 4.6 (p=0.031)
CRP (mg/L)	3.87 \pm 0.28	10.76 \pm 2.56 (p=0.368)	20.9 \pm 5.6 (p=0.005)

505.6±142.8 pg/ml; F ratio: 3.971, $p=0.022$, Table 1). Circulating IGF-1 levels were significantly decreased on both postoperative days (PRE levels: 130.9±12.3 vs. 110.6±13.2 vs. 110.5±11.1 ng/ml; F ratio: 3.766, $p=0.031$, Table 1), while no changes were observed in serum levels of IGFBP-3 throughout the experimental period (PRE levels: 1.95±0.14 vs. 1.96±0.13 vs. 1.96±0.14 µg/ml; F ratio: 0.002, $p=0.998$, Table 1). Correlation analyses showed significant associations between the circulating levels of the acute phase proteins and the growth axis hormones, as shown in Table 2 ($p<0.05-0.001$).

DISCUSSION

Several aspects of postoperative endocrine responses have been described after minimally invasive surgery; however, there is little information regarding the effect of laparoscopic cholecystectomy on the growth endocrine axis. In this prospective trial, we evaluated changes in circulating GH/IGF-1 axis proteins in patients who underwent laparoscopic cholecystectomy. The main finding of the study is that even after this minimally invasive surgery, acute phase responses of the growth endocrine axis are induced, as indicated by the postoperative changes in the circulating levels of IL-6, CRP, GH, and IGF-1.

Changes in circulating levels of IL-6 precede the increase in serum concentration of other acute phase proteins, such as CRP, that act as inflamma-

tory mediators.^{14,15,28} The study of these two inflammation biomarkers, and particularly of IL-6, allows direct quantification of the acute phase inflammatory response,²⁸ as IL-6 and IL-1 are major activators of the cell-mediated immune system response. IL-6 production (as well as that of CRP), which seems to be the most indicative of the severity of tissue injury, is normally moderate in a minimally invasive procedure such as laparoscopic surgery.^{13,14,29-34}

In the present study, a gradual increase in the circulating levels of both IL-6 and CRP was observed on postoperative days 1 and 2, which became significant 48 hrs after the laparoscopic removal of the gallbladder, while high positive correlations were also observed between the postoperative levels of these acute phase factors, indicating their similar responses following the laparoscopic surgery. Similarly, transient^{3,33} or sustained^{2,4,35,36} increases in IL-6 and CRP levels have previously been reported after laparoscopic cholecystectomy, indicating the induction of an acute phase inflammatory response following this minimally invasive surgery.

The inflammatory cytokine actions are mediated, at least partially, by indirect changes in the activity of growth promoting hormones such as GH and IGF-1.³⁷ The stress response to surgery is comprised of a number of hormonal changes induced by neural activation of the hypothalamic-pituitary axis. The duration and magnitude of this response is proportional to the tissue

Table 2. Significant correlations revealed between the circulating levels of the growth axis hormones GH, IGF-1, and IGFBP-3, and the acute phase proteins IL-6 and CRP, preoperatively (PRE) and on days 1 and 2 post laparoscopic cholecystectomy

	CRP PRE	CRP Day 1	CRP Day 2	IL-6 Day 1	BP-3 Day 1	BP-3 Day 2
GH Day 2		$r=0.749$ ($p<0.001$)	$r=0.803$ ($p<0.001$)	$r=0.444$ ($p=0.020$)		
IGF-1 PRE	$r=-0.477$ ($p=0.025$)					
IGF-1 Day 1					$r=0.571$ ($p=0.001$)	
IGF-1 Day 2	$r=-0.471$ ($p=0.027$)				$r=0.540$ ($p=0.002$)	$r=0.790$ ($p<0.001$)
BP-3 Day 2		$r=0.431$ ($p=0.040$)	$r=0.484$ ($p=0.012$)	$r=0.395$ ($p=0.041$)		
IL-6 Day 1		$r=0.887$ ($p<0.001$)	$r=0.860$ ($p<0.001$)			

injury and has an overall catabolic effect on stored body fuels.¹⁴ More specifically, IGF-1 is implicated in a vast number of physiological functions related to tissue growth, development, and metabolism.^{20,38-40} Trauma and conventional surgery result in a state of acquired GH resistance, characterized by high GH levels, decreased anabolic response to GH administration, and reduced levels of its main effector, IGF-1.^{14,22,41,42} It is thought that the acute reduction in circulating IGF-1 levels reflects suppression of the GH/IGF-1 axis in response to the acute stress of surgery.^{22,24,43}

In this study, decreased levels of circulating IGF-1 were found on both postoperative days after laparoscopic cholecystectomy. This decrease was accompanied by a transient increase in GH levels on the first postoperative day, indicating possible growth hormone axis suppression similar to that described after conventional surgery. In addition, significant positive correlations were revealed between the postoperative response of acute phase proteins and GH, as well as inverse correlations between CRP and IGF-1 pre- and postoperative levels. Overall, these relationships may reflect a regulatory effect of IL-6 and CRP on growth axis hormones compatible with a state of systemic inflammation and GH resistance.^{37,42} Moreover, the postoperative correlations found between acute phase proteins and IGFBP-3 may suggest a compensatory systemic response of IGFBP-3 to increase the diminished IGF-1 levels via acute inflammation. The influence of anesthesia could contribute to these stress-induced postoperative changes in GH/IGF-1 levels,^{44,45} although a persistent, lasting more than two days, and not transient increase in GH levels has been reported after major surgical trauma.⁴⁴ To our knowledge, this is the first study showing concurrently the acute phase changes in GH/IGF-1 axis hormones after this minimally invasive cholecystectomy and expands on previous evidence that laparoscopic cholecystectomy can induce an acute endocrine stress response.^{7,13}

Alterations in cytokines and endotoxin may decrease GH receptor availability in the liver, leading to reduced levels of circulating IGF-1,⁴⁶⁻⁴⁸ while the decreased levels of IGF-1 may result in increased GH secretion due to the lack of negative (inhibitory) feedback of IGF-1.^{24,41,49} Taking into consideration the acute inflammatory response observed in the

present study, it can be speculated that the GH/IGF-1 responses may represent cytokine-induced anti-anabolic or catabolic effects even after laparoscopic cholecystectomy. Furthermore, neuroendocrine stress response and inflammatory response, activated by afferent impulses from the site of tissue injury to the brain,¹⁴ occur following laparoscopic cholecystectomy.^{26,50} Central administration of IGF-1 can counteract the activity of pro-inflammatory cytokines produced in the brain;^{24,51,52} however, its decreased levels observed in this study are not likely to attenuate a neuro-inflammatory response.

Although it was previously postulated that circulating or locally produced IGF-1 mediates the growth-promoting actions of GH,^{39,53,54} it has since been established that GH and IGF-1 can also act independently of each other.⁵⁵ Furthermore, investigation into IGF-1 and IGFBP-3 has been used for the evaluation of GH/IGF-1 axis disorders.⁵⁶ Biological actions of IGF-1 are modulated by a family of at least six IGFBPs, as they bind IGF-1 and increase its half-life in the circulation.⁵⁷ Most of the circulating IGF-1 is protected from proteolytic degradation by forming a ternary complex with IGFBP-3 and the glycoprotein acid-labile subunit (ALS).⁵⁸ IGFBPs, and particularly IGFBP-3, can modulate, both in the circulation and the extracellular environment, the extent of IGF-dependent effects via the regulation of free IGF-1 concentration and its local bioavailability in the tissue.^{59,60}

After major surgery there are complex and diverse changes in IGF-1 and IGFBPs. Thus, a larger proportion of circulating smaller fractions of the IGF-1/IGFBP-3 complex has been reported after major conventional surgery, possibly as a result of proteolytic modification of IGFBP-3 to increase the bioavailability of IGF-1.^{43,61-63} In the present study, similarly to previous findings reported after laparoscopic colectomy,³⁴ we did not observe any significant changes in the serum levels of IGFBP-3 on the two consecutive postoperative days after laparoscopic cholecystectomy, suggesting that minimally invasive cholecystectomy does not affect the circulating levels of this growth hormone axis protein. Interestingly however, we demonstrated high correlations between IGFBP-3 and IGF-1 levels on both postoperative days, indicating a possible regulatory effect of IGFBP-3 on circulating IGF-1 postoperatively.

In conclusion, concurrent investigation of the changes in GH/IGF-1 axis hormones after laparoscopic cholecystectomy revealed that this minimally invasive procedure induces acute phase responses in the growth endocrine axis. It remains to be confirmed whether these responses reflect possible cytokine-induced anti-anabolic or catabolic effects even after this minimally invasive surgery.

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