



*Original Article*

## **Increase of serum cortisol levels in response to stress, interleukin-6 (IL-6) levels and adhesion degrees after laparotomy and laparoscopy (experimental research in rabbit which performed ileum abrasion)**

Anangadipa J.<sup>1</sup>, Budijitno S.<sup>2</sup>, Mulyono M.<sup>3</sup>

<sup>1</sup>Surgery Department Resident of Diponegoro University/ Dr. Kariadi Hospital, Semarang

<sup>2</sup>Surgical Oncology Department, Diponegoro University/ Dr. Kariadi Hospital, Semarang

<sup>3</sup>Digestive Surgery Department, Diponegoro University/ Dr. Kariadi Hospital, Semarang

### Abstract

**Background :** Peritoneal adhesion occurs in more than 50 - 97% of cases of transperitoneal surgery which is still an issue of high morbidity and mortality. Minimally invasive surgery has a significantly lower incidence of postoperative peritoneal adhesions. Peritoneal adhesions involve many mediators such as IL-8, TNF- $\alpha$ , IL-1  $\beta$ , IL-6, TGF- $\beta$ . Laparoscopy minimize tissue trauma so that the levels of ACTH, cortisol, prolactin, noradrenaline and adrenaline are lower. This study seeks to prove the correlation between the type of abdominal surgery with peritoneal adhesion levels mediated by the increase in cortisol levels and the increase in IL-6 levels.

**Methods :** A laboratory experimental research using twelve male New Zealand rabbits which divided into two groups was done. The treatments were laparotomy and laparoscopy where the terminal ileum abrasion was done. Blood cortisol levels were taken before treatment and 6 hours after treatment. Levels of IL-6 levels were taken from the peritoneal fluid samples on 7<sup>th</sup> day after treatment. Examination using ELISA method. Assessment of the adhesion degree was done based on macroscopic criteria.

**Results :** The level of serum cortisol, peritoneal fluid IL-6 and intra peritoneal adhesion degrees in the laparotomy group is significantly higher than the laparoscopy group ( $p=0.021$ ,  $p<0.001$ ,  $p=0.002$ ). There were strongly positive and significant correlation between the levels of cortisol and IL-6 ( $p=0.001$  and  $r=0.827$ ). There were strongly positive and significant correlation between the levels of IL-6 and the degrees of adhesion ( $p<0.001$  and  $r=0.878$ ).

**Conclusion :** The level of serum cortisol, peritoneal fluid IL-6 and intra peritoneal adhesion are higher in laparotomy compared with laparoscopy.

**Keywords :** laparotomy, laparoscopy, cortisol, IL-6, peritoneal adhesion.

### **Peningkatan serum kortisol sebagai respons terhadap stres, interleukin-6 (IL 6) dan derajat adhesi setelah laparotomi dan laparoskopi (Penelitian eksperimen pada kelinci dengan gambaran abrasi ileum)**

### Abstrak

**Latar belakang :** Adhesi peritoneal terjadi pada lebih dari 50–97% kasus operasi transperitoneal yang meningkatkan morbiditas dan mortalitas. Operasi invasif minimal akan menurunkan kejadian adhesi peritoneal paska operasi. Adhesi peritoneal melibatkan banyak mediator seperti IL-8, TNF- $\alpha$ , IL-1  $\beta$ , IL-6, TGF- $\beta$ . Laparoskopi akan meminimalkan trauma jaringan sehingga kadar ACTH, kortisol, prolaktin, noradrenalin dan adrenalin lebih rendah. Penelitian ini bertujuan untuk membuktikan hubungan antara tipe operasi abdominal dengan derajat adhesi peritoneal yang diperantarai peningkatan kadar kortisol dan IL-6.

**Metode :** Penelitian eksperimental laboratorium pada 12 kelinci New Zealand dengan abrasi ileum. Sampel dibagi menjadi 2 kelompok, dengan perlakuan yaitu laparotomi atau laparoskopi. Kadar kortisol dan IL-6 diperiksa menggunakan ELISA, kadar kortisol diperiksa sebelum dan 6 jam setelah perlakuan, sedangkan kadar IL-6 diperiksa dari cairan peritoneum 7 hari setelah perlakuan. Penilaian derajat adhesi dilakukan berdasarkan kriteria makroskopik.

**Hasil :** Kadar serum kortisol, IL-6 cairan peritoneum dan derajat adhesi intraperitoneum lebih tinggi pada kelompok laparotomi dibanding laparoskopi (secara berurutan  $p=0,001$ ,  $p<0,001$ , dan  $p=0,002$ ). Terdapat korelasi yang positif antara kadar kortisol dan IL-6 ( $r=0,827$ ;  $p=0,001$ ), dan antara kadar IL-6 dan derajat adhesi ( $r=0,828$ ;  $p<0,001$ ).

## INTRODUCTION

Peritoneal adhesions after abdominal surgery is a serious problem. Occurs in more than 50% to 97% of cases of trans-peritoneal surgery. Adhesion is the cause of high rate of morbidity and mortality. More than 34% of surgical patients re-admitted to hospital because of conditions that directly or may be associated with adhesion. Mortality rates ranged from 4,6 to 13 %. Peritoneal adhesions causing bowel obstruction (*Small Bowel Obstruction*, SBO), re-operation complications, abdominal pain and chronic pelvic, and infertility in women. 65% - 75% of SBO cases occur due to adhesion peritoneal.<sup>1-7</sup> The most common cause of peritoneal adhesions is laparotomy, prolonged surgery, or intraoperative complications include injury to the bowel, bladder, ureters, and bleeding.<sup>6</sup>

Peritoneal adhesion is a process that involves many cells, it has a good vascularization, contains innervation, and mononuclear inflammatory cells could be detected in peritoneal adhesion persistent, not as a process of scarring that is inert and avascular. In an experiment by *Hoshino et al*, demonstrated that macrophages triggered the peritoneal adhesion as an immune response in the peritoneal cavity.<sup>8</sup> As a direct consequence of trauma, peritoneal PMN cells and monocytes migrate into the peritoneum and peritoneal cavity. These cells accumulate several mediators (eg., IL-8, TNF- $\alpha$ , IL-1  $\beta$ , IL-6, TGF- $\beta$ ), which initiated the activation of the extrinsic cascade that results in temporary fibrin matrix that will eventually form the adhesion.<sup>8</sup> In a normal healing process in an intact peritoneum (classical pathway), fibrin which is formed will be degraded into fibrin degradation products through the activities of fibrinolytic system which is powered by *Plasminogen Activator Activity* (PAA) which is located on the sub mesothel blood vessels and mesothel peritoneum. A damage to tissue or blood vessels will lead to low levels of PAA, but will increase the levels of *Reactive Oxygen Species* (ROS) in the blood in response to trauma, so that facilitate the formation of adhesions.

Research by *Ioanna Dimopoulou et al* concluded that in the early phase after major abdominal surgery there was an increase cortisol levels are associated with high levels of ACTH. In the early phase of peritoneal inflammation, cells injury will produce pro inflammatory cytokines produced by cells that are in the peritoneum mesothel and walls of blood vessels, such as interleukin-1

**Simpulan :** Kadar kortisol, IL-6 dan derajat adhesi lebih tinggi pada tindakan laparotomi dibandingkan laparoskopi.

**Kata Kunci :** Laparotomi, laparoskopi, kortisol, IL-6, adhesi peritoneal

(IL-1), IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) which stimulate the adhesions after laparotomy, through the formation of fibrin deposits which will cover the wound. The presence of fibrin stimulates adhesion formation through increased activity stimulated by fibroblast growth factor is *platelet-derived growth factor* (PDGF) and *transforming growth factor- $\beta$*  (TGF- $\beta$ ). Fibroblasts and cells will be deposited mesotel collagen fibers, thus forming a fibrinous adhesion. In this phase, TGF  $\beta$  *Plasminogen Activator Inhibitor* triggered activity (PAI) which inhibits the PAA. After the initial phase of inflammation subsided, there is increased production of anti-inflammatory cytokines, such as IL-10 and IL-4 inhibits the adhesion formation by inhibiting the activity PAI.<sup>2,9</sup>

Surgical approach (open vs. laparoscopic) plays an important role in adhesion formation. In most abdominal procedures, laparoscopic approaches associated with the incidence of postoperative peritoneal adhesions were significantly lower.<sup>1</sup> Since the introduction of laparoscopic approaches, more research on immunological competence and stress response to explain the benefits in minimal invasive techniques.<sup>10</sup> *Brokelman et al* has been demonstrated in a prospective trial that there was no difference between tPA antigen concentration, the activity of tPA, uPA antigen or antigen PAI-1 in peritoneal biopsies were taken at the beginning than at the end of the laparoscopic procedure.<sup>11</sup> Study by *Veenhof et al* suggests that the immune function and inflammatory short term after laparoscopic rectal surgery tend to be better than open surgery.<sup>11</sup>

*Friedrich et al* evaluated the immune response to surgical stress in some patients who underwent laparotomy and laparoscopy and found that ACTH, cortisol, prolactin, noradrenaline and adrenaline were lower in patients who underwent laparoscopy, because tissue trauma is lower on laparoscopic procedure.<sup>13</sup>

Until now there has never been studies linking correlation between the response to stress by the occurrence of postoperative intraperitoneal adhesions laparoscopy and laparotomy. This study seeks to prove the correlation between the type of abdominal surgery in rabbits with peritoneal adhesion levels mediated by the increase in cortisol levels and the increased levels of IL-6.

## MATERIAL AND METHOD

This research is an experimental laboratory test, with a post test design approach that used New Zealand male

rabbits, aged 8–12 weeks weighed between 2500-3000 grams. The experiments were performed with a *simple randomized sampling* method. The groups of rabbit were divided into 2 groups which are group of rabbits that performed laparotomy and laparoscopic groups.

The treatments were conventional laparotomy surgery and abrasion of the terminal ileum using laparoscopic forceps 2 cm long to the oral direction in antimesenterial side, and abrasion of terminal ileum by laparoscopic using 3 ports laparoscopy (one – 11 mm incision and two – 5 mm incision), abrasion of terminal ileum with laparoscopic forceps 2 cm long to the oral direction, antimesenterial side.

Blood cortisol levels are the result of the measurement of cortisol levels taken from blood samples taken before treatment and 6 hours after treatment. Measurements were made with the examination of *enzyme-linked immunosorbent assay* (ELISA), variable scale is ratio.

Levels of IL-6 is the result of measurement of peritoneal fluid levels of IL-6 peritoneal fluid taken from the peritoneal fluid samples on the 7<sup>th</sup> day after treatment using ELISA, the liquid is taken in the pelvic cavity of rabbits ± 1 cc with upstraight position about 15 minutes via laparotomy, variable scale is ratio. The degree of peritoneal adhesion was assessed macroscopically using tables according to *Nair et al.*

## RESULT

The laparotomy group remained 6 rabbits until the end of the study. The results of this group, there were 3<sup>rd</sup> grade intraperitoneal adhesions in 5 rabbits and 4<sup>th</sup> grade in 1 rabbit. In the laparoscopic group, at the end of the study there was 1 dead rabbit on the 5th day and was still remain in the inclusion criteria, and there were 5 rabbits that were still alive until the end of the study. The results in this group, there were 0 grade intraperitoneal adhesions in 4 rabbits, and 1st grade in 2 rabbits.

**TABEL 1**  
Description and normality test cortisol pre and post

Group	Mean ± SD	Median (min – max)	p
<b>Cortisol pre</b>			
Laparotomy	7,28 ± 3,015	7,73 (2,21 – 10,4)	0,583
Laparoscopy	5,13 ± 3,024	4,2 (2,3 – 9,3)	0,207
<b>Cortisol post</b>			
Laparotomy	25,1 ± 4,127	26,3 (17,5 – 29,11)	0,232
Laparoscopy	18,73 ± 3,920	17,75 (14,1 – 23,6)	0,356

**TABEL 2**  
Difference test cortisol pre to post according to laparotomy and laparoscopy group

Group	Cortisol pre	Cortisol post	p
Laparotomy	7,28 ± 3,015	25,1 ± 4,127	0,001*€
Laparoscopy	5,13 ± 3,024	18,73 ± 3,920	< 0,001*€

**TABEL 3**  
Difference test cortisol post according to laparotomy and laparoscopy group

Group	Laparotomy	Laparoscopy	p
Cortisol post	25,1 ± 4,127	18,73 ± 3,920	0,021*£

**TABEL 4**  
**Difference test IL-6 according to laparotomy and laparoscopy group**

Group	Laparotomy	Laparoscopy	p
IL-6	365,57 ± 82,999	143,5 ± 16,908	< 0,001*£

**TABEL 5**  
**Difference test adhesion according to laparotomy and laparoscopy group**

Group	Laparotomy	Laparoscopy	p
Adhesion	3 (3 – 4)	0 (0 – 1)	0,002*¥

**TABEL 6**  
**Correlation test cortisol blood level with peritoneal fluid IL-6 level**

Variable	Mean ± SD	r	p
Cortisol	21,92 ± 5,078	0,827	0,001*§
IL-6	254,53 ± 129,27		

**TABEL 7**  
**Correlation test peritoneal fluid IL-6 level with adhesion degree**

Variable	Mean ± SD	r	p
IL-6	254,53 129,27	0,878	< 0,001*¤
Adhesion	1,75 1,545		

Differentiation test of cortisol pre and post test on each treatment used *Paired t test* ( $p < 0.05$ ) was found significant differences with  $p = 0,001$ . Differentiation test of cortisol post on laparotomy and laparoscopic treatment groups using *independent t test* ( $p < 0.05$ ) found a significant difference ( $p = 0.021$ ).

Based on the descriptive statistical data, levels of peritoneal fluid IL-6 obtained average levels of IL-6 levels in the peritoneal fluid laparotomy group was  $365.57 \pm 82.999$  pg/ml; in the laparoscopy group was  $143.5 \pm 16.908$  ng/ml. Showed average levels of IL-6 in the peritoneal fluid is higher on K2 group than K1 group. Different test levels of IL-6 peritoneum treatment groups laparotomy and laparoscopic treatment groups using *independent t test* ( $p < 0.05$ ) was found significant differences with  $p < 0.001$ .

Based on the degree of adhesion descriptive statistics obtained by the average degree of

intraperitoneal adhesions in laparotomy group was  $3.17 \pm 0.408$ ; in the laparoscopy group was  $0.33 \pm 0.516$ . Showed the average degree of adhesion that is lower in the laparoscopy group compared to laparotomy group. Different test using a bivariate non-parametric *Mann-Whitney test* ( $p < 0.05$ ) showed that all treatment groups had significant difference ( $p = 0.002$ ).

Statistical analysis to determine the correlation between blood cortisol levels which is a numeric variable, with higher levels of IL-6 peritoneal fluid which is also the numerical variables, using *Pearson correlation test*. Correlation test results obtained for the results of the correlation between cortisol variables to IL-6 there is a significant relationship which is very strong positive correlation ( $r = 0.827$ ) between the levels of IL-6 peritoneal fluid and blood cortisol levels in rabbits with  $p = 0.001$ , which means that the high blood cortisol levels, so the levels of peritoneal fluid IL-6 higher.

Statistical analysis to determine the correlation between the levels of IL-6 peritoneal fluid which is a numeric variable, the degree of intraperitoneal adhesions that are ordinal variables, using the *Spearman correlation test*. Correlation of test results obtained are very strong negative correlation ( $r = 0.878$ ) between the levels of IL-6 peritoneal fluid and the degree of intraperitoneal adhesions in rabbits with  $p < 0.001$ , which means that the higher levels of IL-6 peritoneal fluid, then the degree of intraperitoneal adhesion higher.

From the analysis above it can be concluded that laparoscopy will reduce the degree of intraperitoneal adhesions and reduce levels of peritoneal fluid IL-6 significantly compared with treatment group laparotomy and has a very strong positive correlation.

## DISCUSSION

Intraperitoneal adhesion formation experimentally can be done through various ways, namely ischemia models, model of peritoneal injury, thermal injury models, with foreign objects, with chemicals and bacterial.<sup>14</sup> Ileum abrasion in this experiment included in the model of peritoneal injury. This method was chosen because of injuries caused by abrasion injury resembling the peritoneal injury when either abdominal surgery with laparoscopic techniques or laparotomy.

Based on the degree of adhesion descriptive statistics obtained by the average degree of intraperitoneal adhesions in laparotomy group was  $3.17 \pm 0.408$ ; in the laparoscopic group was  $0.33 \pm 0.516$  which indicates that the abrasion of the ileum method is quite adequate to cause intraperitoneal adhesions.

This study proves that laparoscopy cause lower stress responses are assessed from blood cortisol levels, leading to decreased levels of IL-6 peritoneal fluid and decrease the degree of intraperitoneal adhesions compared with laparotomy, the rabbit ileum abrasion done by laparotomy and laparoscopy.

Research carried out, from 12 rabbits, six rabbits were laparotomy can survive until the end of the study so that it can be analyzed and from 6 rabbits were performed laparoscopically 5 animals can survive, 1 tail die on day 5 (entry criteria inclusion), so that the analysis can be done entirely.

Laparoscopy is associated with reduced tissue trauma that is also related to the low systemic stress response.<sup>10</sup>

Surgical trauma stimulates a series of hormonal and metabolic changes that the stress response. Operations also induces neurohormonal events that initially includes activation of the sympathetic nervous system and stimulation of the hypothalamic - pituitary - adrenal axis. Then the adrenal cortex is activated, promotes the release of neurohormonal transmitter that will affect postoperative pain intensity and duration of

postoperative ileus. ACTH, catecholamines, cortisol, and glucagon all play an important role in mediating the stress response, which in sepsis and trauma, catecholamines, cortisol, and glucagons released in large quantities.<sup>15</sup>

Injury to the lining of the peritoneal mesothel cells will result in the release of various cytokines and proinflammatory mediators by peritoneal mesothelium cells and vascular endothelial injured, as a result of the production of pro-inflammatory cytokines that will stimulate the activity of the blood coagulation cascade system and is an important component of the systemic inflammatory response.<sup>16</sup>

The results of the statistical test of correlation between blood cortisol levels and levels of IL-6 peritoneal fluid, obtained are meaningful relationships very strong positive correlation between the levels of IL-6 peritoneal fluid and blood cortisol levels in rabbits made intraperitoneal adhesions, which means the higher blood cortisol levels, the higher of peritoneal fluid IL-6 levels.

The result of statistical test to the correlation between the levels of IL-6 peritoneal fluid and intraperitoneal adhesion degree, obtained a very strong positive correlation between the levels of peritoneal fluid IL-6 and the degree of intraperitoneal adhesions in rabbits made intraperitoneal adhesions, which means that the higher levels of IL-6 fluid peritoneum, then the higher the degree of intraperitoneal adhesions.

Laparoscopy, reduce the degree of adhesion very significant compared with laparotomy in rabbits do illeum abrasion, it can be proved in laparoscopy potentiating suppress effect of IL-6 peritoneal fluid. Intraperitoneal adhesion process is influenced by three main components that interact with each other, namely inflammation, fibrinolysis and extracellular matrix remodeling. Increased levels of IL-6 will be followed by an increase in the degree of adhesion. This was evident in this study, where there is a significant correlation with the direction of a very strong positive correlation.

## CONCLUSION

In this study it was concluded in the form of blood cortisol levels in rabbit ileum abrasion laparotomy was significantly higher than that performed laparoscopically abrasion rabbit ileum. Levels of IL-6 in the peritoneal fluid of rabbits were performed laparoscopic ileal abrasion significantly lower than the laparotomy abrasion rabbit ileum. The degree of intraperitoneal adhesions in the rabbit ileum abrasion laparotomy was significantly higher than that performed laparoscopically abrasion rabbit ileum. There is a significant correlation between increased levels of cortisol blood levels of IL-6 in the peritoneal fluid were performed abrasion rabbit ileum, with relationship is very strong positive correlation. There is a significant correlation between

increased levels of IL-6 peritoneal fluid with increasing degrees of intraperitoneal adhesions in rabbits conducted abrasion ileum, with a very strong positive correlation.

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