SHORT PAPER

The Relationship between Plasma Levels of Interleukin-6, Multiple Organ Dysfunction and Mortality in Orthopedic Patients

Adel Ebrahimpour[1], Ahmadreza Mirbolook[2], Mohammadali Okhovatpour[1], Mohammad Reza Minator Sajjadi[1], Kamyar Makvandi[1], Mohammad Sadegh Mousavi[2], Sepehr Saghary[2], Mehrdad Sadighi[1*]

[1]Taleghani Hospital, Research Development Committee, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, [2]Poursina Hospital, Guilan University of Medical Sciences, Rasht, Iran

ABSTRACT

Background: Interleukin 6 (IL-6) functions as both a pro-inflammatory cytokine and an anti-inflammatory cytokine. **Objective:** To evaluate the levels of IL-6 in patients with multiple organ dysfunction syndrome (MODS). **Methods:** Level of IL-6 was assessed and recorded for 14 days subsequent to the injury in 161 multiple trauma patients. MODS were diagnosed using Marshal Score. Injury Severity Scoring (ISS) was measured for all patients. **Results:** The results of this study indicated that there was a significant relationship between the level of IL-6 and ISS on the post trauma days number one and two (P=0.0001). The high level of IL-6 on the post trauma day number 2 was associated with high mortality rate. **Conclusion:** Our study suggests the second day as the golden time for measuring the serum levels of IL-6. These findings warn us to take more health care actions in patients with higher serum levels of IL-6 on the second day.

Sadighi M, et al. Iran J Immunol. 2018; 15(2):156-164.

Keywords: IL6, Mortality Rate,Multiple Organ Dysfunction, Multiple Trauma, Prognosis

---

*Corresponding author: Dr. Mehrdad Sadighi,Taleghani Hospital, Research Development Committee, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran, e-mail: mehrdad_1330@yahoo.com

INTRODUCTION

The number of trauma-related deaths has dropped over the past decade thanks to the improvements in medicine (1). Multiple Organ Dysfunction Syndrome (MODS) is still considered as a common clinical complication leading to death following severe trauma. Although the survival rate of patients with multiple injuries has improved over the recent years, the rate of organ dysfunction is yet to change (2,3). At present, the rate of mortality in patients with MODS (caused by severe injuries) is more than 50 percent (4,5,6). Accordingly, for a timely treatment, it is crucial to identify high-risk patients after trauma. Research has revealed that the immunological changes following trauma result in MODS or, worse yet, death. Even though external supports such as ventilators, dialysis machines, and inotropic drugs compensate for patients' internal functional defects, the final results are still unpredictable (7,8). It is quite demanding to carry out a preliminary assessment of prognosis in patients suffering from multiple injuries. The clinical conditions of such patients are traditionally checked through evaluating the cardio-vascular performance, and kidney, liver, and respiratory functions (9). The predictive value of several clinical parameters is uncertain. For instance, it has been shown that initial lactate level is associated with MODS and long-term hyperlactatemia is related to increased mortality rate (10,11). Evaluating clinical status and prognosis is still major obstacle during the treatment course of patients with multiple injuries. Clinical parameters such as blood pressure, PH or heart rate have proven unsuccessful in assessing post-trauma situation (12). Many clinical studies have demonstrated that the augment in the level of plasma cytokines is a concomitant of MODS, injury severity score, and mortality (13-18). The relationship between high levels of IL-6 and clinical outcome in the pediatric patients with serious head injuries is significant. Moreover, increased amount of IL-6 is considered as a predictor for nosocomial infections in trauma patients (17,18). While plasma levels of IL-6 can predict the outcomes in murine sepsis model, data in human models differ over the predictive power of IL-6 (19-22). Overall, the present research aimed at assessing the relationship between plasma levels of IL-6 with MODS and mortality, and to further specify the threshold effect for MODS spread. The hypothesis is that high plasma levels of Il-6 can be a predictor for MODS and mortality following severe trauma.

MATERIALS AND METHODS

Study Population. Out of the 161 patients with trauma-related dysfunctions in multiple organs, 96 (59.6 %) were male and 65 (40.4 %) were female. The average age range of patients was 39.28 ± 9.23 years. Patients with multiple injuries and severity score >16, hospitalized in the ICU of Rasht Poursina Hospital, were selected as the participants of this study conducted from January to December of 2013.The patients aged 16 to 65 years old. The excluding criteria were patients discharged (earlier than 14 days) from hospital with good general conditions, patients with a history of steroid consumption, and anti-inflammatory or hormone therapies, and those with malignancy or chronic diseases of vital organs such as liver, kidneys or lungs. An informative consent was signed either by the patients or their relatives. Additionally, Abbreviated Injury Scale (AIS) was identified after trauma CT scan (of head, cervical spine, thorax, abdomen and pelvis) and the severity of injury was determined via Injury Severity Scoring (ISS).

Clinical Parameters and Evaluation Results. Patients' plasma were carefully collected on the first, second, third, fourth, seventh, tenth and fourteenth days and examined based on the manufacturer instructions using IL-6 quantification Immulite ® System: Random Access Immunoassay Analyzer; DPC-Biermann, Bad Nauheim, Germany. The IL-6 antigen levels of all participants were measured in 100µl plasma through the use of both a sequential immunometric assay and Immulite analyzer. The lowest detection limit of this system is 5 pg/ml which was consequently used as the cutoff for the statistical analyses. Levels higher than the cutoff were reported herein as higher than 5 pg/ml, high, raised, or elevated. The results of IL-6 were recorded up to 14 days after the injury. Sepsis was diagnosed on two consecutive days based on Consensus Conference of the American College of Chest Physicians (ACCP) and Society of Critical Care Medicine (SCCM) classification (1). MODS was diagnosed by Marshal score (2), proven to be the most reliable score to diagnosis MODS (3). MODS was diagnosed only when a score >12 was recorded on two consecutive days or at least for three days during the observation period (4).

Management and Treatment. After admission, all patients received an arterial and a central venous line. A standardized clinical examination, accurate assessment with FAST (Focused Assessment with Sonography for Trauma), was performed and at least imaging of the chest and pelvis was taken. Following diagnosis in the emergency room, trauma-related CT scans (CT scan of head, cervical spine, chest, abdomen and pelvis) were performed. Results were analyzed by radiology and trauma surgery attendants. At the time of admission to ICU, clinical examination and FAST were repeated. All the studied patients were treated by physicians exclusively for the purpose of the present research.

Statistical Analyses. Based on the daily records, the statistically significant difference levels of IL-6 from the date of injury up to 14 days after the injury was evaluated using ANOVA, student's *t test*, Pearson correlation and the Chi-square tests. P values lower than 0.05 were considered as statistically significant. Data processing and statistical analyses were done by use of SPSS version 17.0 software (SPSS, Chicago, Ill).

RESULTS AND DISCUSSION

The AIS scores of versions 1, 2, 3, 4, 5 and 6 were 2.96±0.8, 2.63±0.72, 3.02±0.84, 0.1±0.3, 2.57±0.81 and 1.52±1.01, respectively. Overall, the mean ISS score was recorded as 29.24 ± 7.44 and the MODS score was 13.84±4.65. In total, 25/161(15.52%) patients decreased. Figure 1 shows different levels of IL-6 divided by the studied times where, as can be observed, the highest level (251.11± 31.54 Pico grams/ml) belongs to the first 24 hours, while the minimum (85.44± 19.05 Pico grams/ml) occurs seven dayspost trauma. Figure 2 specifies that the high ISS had significant relationship with the level of IL- 6 on the first and second days after trauma (Sensitivity= 100% & Specificity= 79.1%) (p=0.0001). The levels of IL-6 at other times of measurement (three, four, seven, ten, and fourteen days following the trauma) were p=0.634, p=0.953, p=0.071, p=0.921 and p=0.213, respectively. Figure 3 shows that the high MODS score had significant association with the level of serum IL-6 on the second day post trauma (p=0.0001).

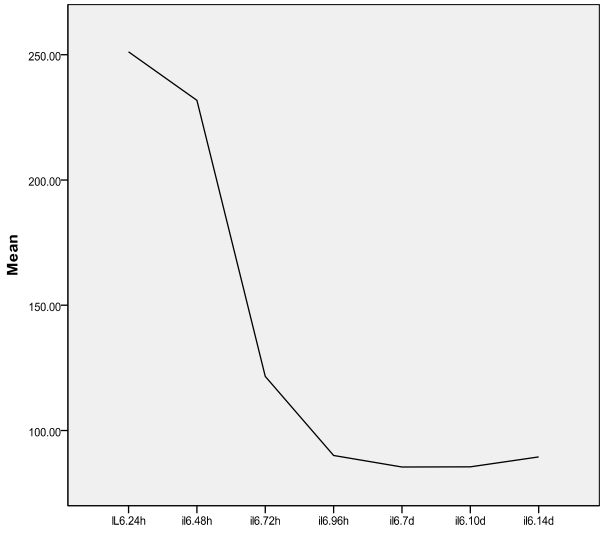

Figure 1. Different levels of IL-6 from date of injury up to 14 days after injury. The highest level of IL-6 was in the first 24 hours 251.11± 31.54 Pg/ml and its lowest level was on the post trauma on the day number seven 85.44± 19.05 Pg/ml.

At other times of measurement (days one, three, four, seven, ten and fourteen post trauma), the relationship between IL-6 plasma levels and MODS score was P=0.058, P=0.973, P=0.256, P=0.849, P=0.634 and P=0.755, respectively (Sensitivity= 97.9% & Specificity= 74.6%).

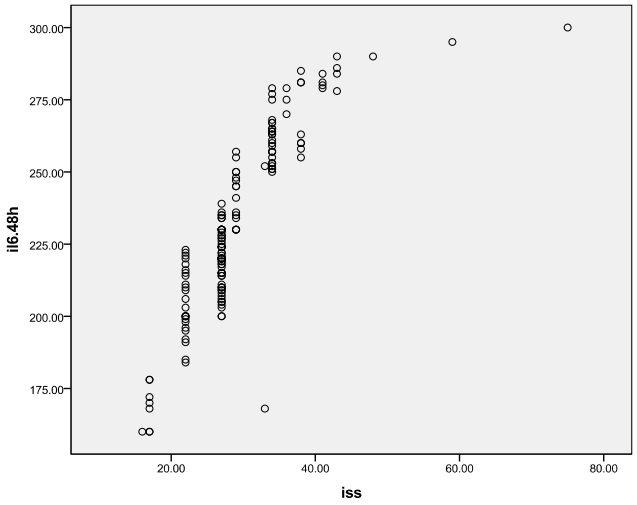

Figure 2. The high ISS had significant relationship with the level of IL- 6 on the post trauma on the day number one and two (Sensitivity= 100% & Specificity= 79.1%) (P=0.0001). The levels of IL-6 on post trauma day number three, four, seven, ten, and fourteen was P=0.634, P=0.953, P=0.071, P=0.921 and P=0.213, respectively.

Table 1 illustrates the distribution frequency of IL-6 levels divided by mortality whereon day two the IL-6 level has a significant association with the frequency of deaths (Sensitivity= 100% & Specificity= 80.9%). According to the Table 2, the 8 patients who developed sepsis had significant levels of IL-6, 24 and 48 hours post injury (P=0.022 & P=0.024 respectively).

Based on different studies on elective surgeries, it was found out that levels of IL-6 might reflect the severity of surgical trauma and damage to the underlying tissue. ISS values were determined based on the final assessment of each patient during the first day of trauma. The obtained results partly corroborated the hypothesis that IL-6 level is

a preliminary variable for explaining the extent of tissue damage, determined by a timely measurement of IL-6 levels. Certain studies have demonstrated that even patients who died shortly after trauma had high levels of IL-6 for several hours (29).

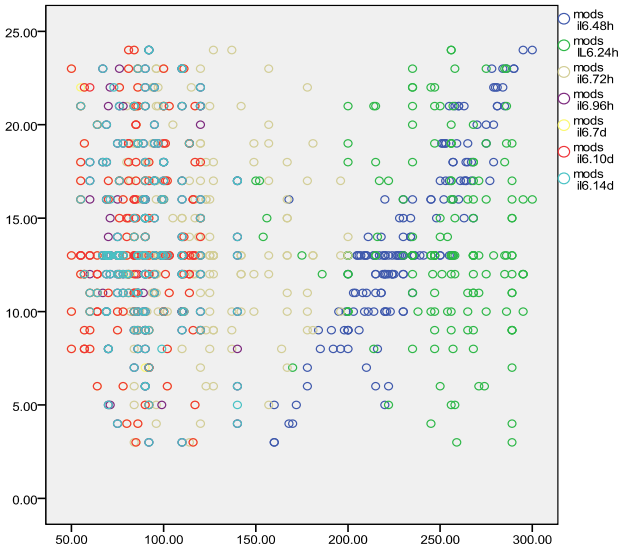

Figure 3. The high MODS score had significant association with the level of IL-6 on the post trauma day number two (P=0.0001). While the level of IL-6 on post trauma day number one, three, four, seven, ten and fourteen were P=0.058, P=0.973, P=0.256, P=0.849, P=0.634 and P=0.755, respectively.

In other words, it appears that multi-organ failure and the following death are significantly associated with increased levels of IL-6 belonging to the second day. Recent literature supports our findings and signifies a continuous reduction in the density of IL-6 after an initial increase (23,24,30).

Table 1. The levels of IL-6 (Pg/mL) among the mortality group during 24 hr, and 4 days. The data interval showed significant increasing level of IL-6 at 48 hours post injury (p<0.0001).

Time Points	Mortality	N	Mean	Std. Deviation	P value
IL6.24h	Yes	25	250.24	21.96	0.8813
	No	136	251.27	33.07	
IL6.48h	Yes	25	276.84	12.51	<0.0001
	No	136	223.53	25.33	
IL6.72h	Yes	25	115.12	26.29	0.2586
	No	136	122.78	31.82	
IL6.96h	Yes	25	93.24	17.22	0.3733
	No	136	89.52	19.47	
IL6.7d	Yes	25	83.32	18.97	0.5449
	No	136	85.84	19.11	
IL6.10d	Yes	25	83.52	18.68	0.5715
	No	136	85.87	19.11	
IL6.14d	Yes	25	90.88	17.41	0.6966
	No	136	89.24	19.61	

Although the results of studies regarding IL-6 were retrieved based on elective trauma surgery, the findings related to patients with Carcinoma (28), and those undergone Cholecystectomy were consistent with our results (29).

Table 2. The levels of IL-6 (Pg/mL) among the 8 patients who developed sepsis. The Data showed significant increasing level of IL-6 at 24 and 48 hours and 10 days post injury (P=0.0244, P=0.0222 and P=0.0495, respectively).

Time Points	Sepsis	N	Mean	Std. Deviation	P value
IL6.24h	Yes	8	275.50	31.02	0.0244
	No	153	249.84	31.15	
IL6.48h	Yes	8	255.88	45.42	0.0222
	No	153	230.55	29.36	
IL6.72h	Yes	8	112.25	26.31	0.3847
	No	153	122.08	31.30	
IL6.96h	Yes	8	95.75	9.82	0.3921
	No	153	89.79	19.47	
IL6.7d	Yes	8	89.00	13.92	0.6797
	No	153	91.84	19.13	
IL6.10d	Yes	8	96.04	12.81	0.0495
	No	153	82.51	19.08	
IL6.14d	Yes	8	91.75	9.86	0.8212
	No	153	90.17	19.57	

Via the current research, it was attempted to find out whether or not IL-6 can be a valuable predictor for injury and initial damages after trauma and ISS and if IL-6 has any association with mortality rate, MODS and the overall prediction of late complications in traumatic patients. In their study, Gebhard *et al.* (30) stated that serum level of IL-6 can be considered as an indicator for the outcome and complications of trauma. They further suggested that the level of IL-6 definitely gives a primary variable for injury severity, hence an important factor for specifying the first step of treatment in trauma surgery. Previous studies have expansively proven the effect of IL-6 in predicting the severity of initial damage after trauma. However, its relationship with MODS and mortality (delayed effects) has been the subject of controversy (31,32).

To answer the two raised questions, the level of IL-6 was regularly measured for 1-14 days, sequentially. The results of the present study demonstrated that the mean of IL-6 in the first 24 hours can predict the ISS all by itself. However, it showed no significant relationship with MODS and mortality and was not a valuable clue in the first 24 hours.

On the second day, the level of IL-6 was significantly associated with ISS, Sepsis, MODS, and mortality, hence considered as a valuable clinical clue on the second day. No relationship was found between the delayed and early parameters from the third day on.

In the present research, IL-6 was identified as one of the reactive cytokines of complications associated with trauma and not merely a measuring tool for injury severity and early management of trauma. Martin et al. showed that the increased IL-6 plasma levels are associated with morality in the stage of septic shock (28). Moreover, the increased amount of IL-6 in AIS was also a predictor of nosocomial infections in trauma patients. The difference between the present study and Gebhard's study is that

we measured IL6 gradually and over different time intervals, finding that although the highest serum levels of IL-6 were observed on the first day, second day was the optimal time for the prediction of injury severity based on ISS, MODS, and morality (specificity 100%, 100%, and 97.9%, respectively). Moreover, day two is the best time for measuring the serum levels of IL-6, hence the fact that more health care actions must be taken on patients with higher serum levels of IL-6 on the second day. Several cytokines such as IL-10 have a role in inflammation response; it has been shown that IL-6 is an anti-inflammation regulator stimulating potent anti-inflammation cytokines such as IL-10 (33). Sapan *et al.* reported patients with very severe injuries (ISS ≥30) had augmented IL-6 and IL-10 levels. In no survivor groups, however, these cytokine levels declined (34). The present findings were in accordance with Sapan although IL-10 level was not investigated in this study. In addition to IL-6, there are other factors determining MODS. Polymorphism and genomic variants including gene expression profile in trauma require further studies for a more clear understanding as to the body's response to injury. In conclusion, our findings revealed that high levels of IL-6 are associated with poor prognosis on the second day post-trauma.

ACKNOWLEDGEMENTS

This work was supported by Orthopedic Research Center, Guilan University of Medical Sciences, Rasht, Iran.

REFERENCES

1. Abbasi H, Bolandparvaz S, Yadollahi M, Anvar M, Farahgol Z.Time distribution of injury-related in-hospital mortality in a trauma referral center in South of Iran (2010-2015). Medicine (Baltimore). 2017; 96:e6871.
2. Larsen P, Goethgen CB, Rasmussen S, Iyer AB, Elsoe R. One-year development of QOL following orthopaedic polytrauma: a prospective observational cohort study of 53 patients. Arch Orthop Trauma Surg. 2016;136:1539-1546.
3. Caragounis EC, Fagevik Olsén M, Pazooki D, Granhed H. Surgical treatment of multiple rib fractures and flail chest in trauma: a one-year follow-up study. World J Emerg Surg. 2016;11:27.
4. Shin J, Choi Y, Lee SG, Kim W, Park EC, Kim TH.Relationship between socioeconomic status and mortality after femur fracture in a Korean population aged 65 years and older: Nationwide retrospective cohort study. Medicine. 2016;95:e5311.
5. Vaidya R, Scott AN, Tonnos F, Hudson I, Martin AJ, Sethi A.Patients with pelvic fractures from blunt trauma. What is the cause of mortality and when? Am J Surg. 2016; 211:495-500.
6. Lin JC, Liang WM.Mortality and complications after hip fracture among elderly patients undergoing hemodialysis. BMC Nephrol. 2015;16:100.
7. Patka P.Damage control and intramedullary nailing for long bone fractures in polytrauma patients. Injury. 2017;48 Suppl 1:S7-S9.
8. Jansen G, Leimkühler K, Mertzlufft F. Intramedullary placement of intraosseous cannulas inserted in the preclinical treatment of polytrauma patients: A retrospective, computed tomography-assisted evaluation. Anaesthesist. 2017;66:168-176.
9. Gandhi RR, Overton TL, Haut ER, Lau B, Vallier HA, Rohs T, et al. Optimal timing of femur fracture stabilization in polytrauma patients: A practice management guideline from the Eastern Association for the Surgery of Trauma. J Trauma Acute Care Surg. 2014;77:787-795.
10. Lawton L, Crouch R, Voegeli D.Is lactate an effective clinical marker of outcome for children with major trauma? - A literature review. Int Emerg Nurs. 2016;28:39-45.

11. Allwell-Brown E, Afuwape OO, Ayandipo O, Alonge T.Correlation of the association of serum lactate, random blood sugar, and revised trauma score as predictors of outcome in hemodynamically unstable abdominal emergencies.Niger J Clin Pract. 2016;19:196-200.
12. Waydhas C, Nast-Kolb D, Trupka A, Zettl R, Kick M, Wiesholler J, et al. Posttraumatic inflammatory response, secondary operations,and late multiple organ failure. J Trauma. 1996;40:624-630.
13. Paine R 3rd, Standiford TJ, Dechert RE, Moss M, Martin GS, Rosenberg AL, et al.A randomized trial of recombinant human granulocyte-macrophage colony stimulating factor for patients with acute lung injury. Crit Care Med. 2012; 40:90-7.
14. Liu FC, Tsai YF, Tsai HI, Yu HP.Anti-Inflammatory and Organ-Protective Effects of Resveratrol in Trauma-Hemorrhagic Injury.Mediators Inflamm. 2015;2015:643763.
15. Du CG, Zhang YZ, Chen W. Progress in diagnosis and treatment of ipsilateral femoral neck and shaft fracture .Zhonghua Wai Ke Za Zhi. 2016;54:553-7.
16. Partrick DA,Moore FA, Moore EE,Biffl WL, Sauaia A, Barnett CC Jr. The inflammatory profile of interleukin-6, interleukin-8, and soluble intercellular adhesion molecule-1 in postinjurymultipleorgan failure. Am J Surg. 1996; 172:425-429.
17. Roumen RM,Hendriks T, van der Ven-Jongekrijg J,NieuwenhuijzenGA,Sauerwein RW, van der Meer JW, et al.Cytokine patterns inpatients after major vascular surgery, hemorrhagic shock,and severe blunt trauma. Relation with subsequent adultrespiratory distress syndrome and multiple organ failure. Ann Surg. 1993; 218:769-776.
18. Frink M,Pape HC, van Griensven M,Krettek C,Chaudry IH, Hildebrand F. Influence of sex and age on mods and cytokines after multiple injuries. Shock. 2007; 27:151-156.
19. Naffaa M, Makhoul BF, Tobia A, Kaplan M, Aronson D, Saliba W, et al.Interleukin-6 at discharge predicts all-cause mortality in patients with sepsis. Am J Emerg Med. 2013; 31:1361-4.
20. Bahrami S,Redl H, Yao YM,Schlag G. Involvement of bacteria/endotoxin translocation in the development of multiple organ failure. Curr Top MicrobiolImmunol. 1996;216:239-258.
21. Law MM, Cryer HG, Abraham E. Elevated levels of soluble ICAM-1 correlate with the development of multiple organ failure in severely injured trauma patients. J Trauma. 1994; 37:100-109.
22. Casili G, Impellizzeri D, Cordaro M, Esposito E, Cuzzocrea S. B-Cell Depletion with CD20 Antibodies as New Approach in the Treatment of Inflammatory and Immunological Events Associated with Spinal Cord Injury. Neurotherapeutics. 2016;13:880-894.
23. Doyle SL, Mongan AM, Donohoe CL, Pidgeon GP, Sherlock M, Reynolds JV, et al.Impact of visceral obesity and metabolic syndrome on the postoperative immune, inflammatory, and endocrine response following surgery for esophageal adenocarcinoma. Dis Esophagus. 2017;30:1-11.
24. Horikoshi Y, Goyagi T, Kudo R, Kodama S, Horiguchi T, Nishikawa T.The suppressive effects of landiolol administration on the occurrence of postoperative atrial fibrillation and tachycardia, and plasma IL-6 elevation in patients undergoing esophageal surgery: A randomized controlled clinical trial. J Clin Anesth. 2017;38:111-116.
25. Facy O, Paquette B, Orry D, Santucci N, Rat P, Rat P, et al. Inflammatory markers as early predictors of infection after colorectal surgery: the same cut-off values in laparoscopy and laparotomy? Int J Colorectal Dis. 2017;32:857-863.
26. Mari G, Crippa J, Costanzi A, Mazzola M, Rossi M, Maggioni D. ERAS Protocol Reduces IL-6 Secretion in Colorectal Laparoscopic Surgery: Results From a Randomized Clinical Trial. Surg Laparosc Endosc Percutan Tech. 2016;26:444-448.
27. Gomatos IP, Alevizos L, Kalathaki O, Kantsos H, Kataki A, Leandros E, et al.Changes in T-lymphocytes' viability after laparoscopic versus open cholecystectomy. Int Surg. 2015;100:696-701.
28. Schietroma M, Colozzi S, Pessia B, Carlei F, Amicucci G. The Effects of High-Concentration Oxygen on Inflammatory Markers in Laparoscopic Cholecystectomy: A Randomized Controlled Trial. Surg Laparosc Endosc Percutan Tech. 2017;27:83-89.
29. Weis F, Beiras-Fernandez A, Schelling G, Briegel J, Lang P, Hauer D, et al.Stress doses of hydrocortisone in high-risk patients undergoing cardiac surgery: effects on interleukin-6 to interleukin-10 ratio and early outcome. Crit Care Med. 2009; 37:1685-90.

30. Okeny PK, Ongom P, Kituuka O.Serum interleukin-6 level as an early marker of injury severity in trauma patients in an urban low-income setting: a cross-sectional study.BMC Emerg Med. 2015;15:22.
31. Yang S, Hu S, Hsieh YC, Choudhry MA, Rue LW 3rd, Bland KI, et al.Mechanism of IL-6-mediated cardiac dysfunction following trauma-hemorrhage.J Mol Cell Cardiol. 2006; 40:570-9.
32. Lenz A, Franklin GA, Cheadle WG. Systemic inflammation after trauma.Injury. 2007; 38:1336-45.
33. Jawa RS, Anillo S, Huntoon K, Baumann H, Kulaylat M. Interleukin-6 in surgery, trauma,and critical care part II: clinical implications. J Intensive Care Med. 2011; 26:73-87.
34. Sapan HB, Idrus P, Irawan J, Ilhamjaya P, Muh NM, Aryono DP, et al. Pattern of cytokine (IL-6 and IL-10) level as inflammation and anti-inflammation mediator of multiple organ dysfunction syndrome (MODS) in polytrauma. Int J Burn Trauma. 2016;6:37-43.