IJBCS
ISSN: 2147-1428

Original Article
International Journal of Basic and Clinical Studies (IJBCS)
2013;1(1): 50-71. Hakimoglu Y et al.

The Effect of Nitrous Oxide on IL-6, TNF-α, and IL-10 Levels

Yasemin Hakimoglu[1], Murat Can[2], Sedat Hakimoglu[3], Ayca Gorkem Mungan[2]

Sereften Acikgoz[2], Necmettin Aydin Mungan[4], Isil Ozkocak Turan[5]

[1]M.D. Hatay Antakya State Hospital, Department of Biochemistry, Hatay, Turkey
[2]Associate Prof. M.D. Bulent Ecevit University, Medical Faculty, Department of Biochemistry, Zonguldak, Turkey
[3]Assist.Prof. M.D. Mustafa Kemal University, Medical Faculty, Department of Anesthesiology and Reanimation, Hatay, Turkey
[4]Prof. M.D. Bulent Ecevit University, Medical Faculty, Department of Urology, Zonguldak, Turkey
[5]Prof.M.D. Bulent Ecevit University, Medical Faculty, Department of Anesthesiology and Reanimation, Zonguldak, Turkey

Corresponding Author: Yasemin Hakimoglu
Hatay Antakya State Hospital, Department of Biochemistry, Hatay, Turkey
E-mail address: dr.yasemin61@hotmail.com

Abstract

In our study, we aimed to investigate the effects of anesthetic agent nitrous oxide on inflammatory and antiinflammatory parameters. Forty-four patients undergoing elective urological surgey were included in the study. Anesthesia maintenance provided with 1-2 MAC sevoflurane, O_2 50%, N_2O 50% in 4L/m transporter gase for group 1 and 1-2 MAC sevoflurane, O_2 50%, air 50% in 4L/m transporter gase for group 2. Venous blood samples for the measurement of IL-6, TNFα, IL-10 were taken before the induction of anaesthesia, 60 minutes of anesthesia induction, at the end of anaesthesia and 24 hours after operation. In

statistical analysis Bonferroni test and analysis of variance at the repeated measures were used. In both groups serum IL-6 levels was significantly increased in the postoperative period. Whereas in this period TNF-α levels were significantly decreased. The rate of increase for IL-6 and rate of decline for TNF-α were found higher in nitrous oxide group. IL-10 levels did not show a significant change in both groups. In conclusion, nitrous oxide showed significant effect on inflammatory and antiinflamatory parameters. Further detailet studies are required to evaluate the effect of nitrous oxide.

Keywords: Nitrous oxide, IL-6, TNF-α, IL-10

Introduction

Many normal functions of the immune system are depressed after exposure to the combination of anaesthesia and surgery (1). Surgery and trauma induce alterations in both immune responsiveness and organ function (2,1) .Impairment of the immune response by both anaesthesia and surgery is suggested by clinical observations of both the high rate of infection seen in postoperative patients (3) and bone marrow depression after prolonged exposure to anaesthesia (4). The principal immunological deficit after trauma and major surgery is decreased cell mediated immunity from an impaired natural killer (NK) cell response and T helper (Th)1 lymphocyte development, which probably results in preferential Th2 development (5).

Anesthesia and surgical intervention, leads to the development of systemic inflammatory response (6). Proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-2, IL-6, and IL-8 are important mediators of host defense mechanisms and the systemic inflammatory response and they may be related in part to postoperative complications (7). Cytokines are a heterogeneous group of proteins, variously termed

lymphokines, monokines, interleukins and interferons, which act on cell-surface receptors to regulate and modify cell growth, maturation and repair (5). Cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma. Some cytokines act to make disease worse (proinflammatory), whereas others serve to reduce inflammation and promote healing (anti-inflammatory).

Interleukin (IL)-6 is a pleiotropic cytokine whose actions include modulation of proliferation, differentiation, and maturation of hemopoietic progenitors and other cell lineages; growth regulation of certain carcinoma cell lines; and control of cellular metabolic activities. Recently, a number of clinical studies have described the IL-6 response to trauma, bums, and elective surgery. Although IL-6 is considered an integral mediator of the physiologic acute phase response to injury, excessive and prolonged postinjury elevations of circulating IL-6 levels are associated with morbidity and mortality (8).

TNF-α is a primary inflammatory mediator that is responsible for many physiological changes such as hypotention, fever, tachycardia, oliguria and changes in consciousness in patients with septic shock (9) It has been reported that IL-10, a well-characterized natural antiinflammatory cytokine, regulates the function of several cell types (10,11). The severity of the inflammatory response depends on the pharmacological effects of anesthetic agents and duration of anesthesia (6).

Nitrous oxide (N_2O; laughing gas) has been widely used in clinical practice for decades because its effective analgesic properties are achieved at concentrations below those required for general anesthesia (12). Nitrous oxide is colorless, odorless to sweet-smelling, and nonirritating to the tissues. It is nonflammable but will support combustion (13). These analgesic effects, coupled with a rapid onset and short duration of action, have made nitrous oxide the oldest inhalational anesthetic in clinical anesthesia and analgesia (12). Nitrous oxide

also interferes with the immune system, and infections following anesthesia are attributed to this effect. Although controversial, in vitro as well as in vivo studies have revealed decreased neutrophil motility and chemotaxis as well as impairment of the leukocyte oxidative response in response to nitrous oxide (14).

In our study, we aimed to investigate the effects of anesthetic agent nitrous oxide on inflammatory and antiinflammatory parameters.

Materials and Methods

Official approval, dated 15.01.2009 and numbered 2009/01/15, to the effect that the present study is in compliance with ethical principles was obtained from the Ethical Committee at Karaelmas University Application and Research Hospital.

44 ASA I-III adult patients who were between 18 and 65 and were to undergo an elective urological surgical initiative that would last 1 to 4 hours in the main operation room at Karaelmas University Application and Research Hospital were included in this study between January 2009 and January 2010. Patients with chronic metabolic diseases, liver failures and acute anemia were not included in the study. All the patients were applied the standard İM midazolam (0.07 mg/kg) premedication 1 hour before the surgery. Blood samples were taken from the patients prior to anesthesia in order to determine their TNFα, IL-6, IL-10 levels. Before induction, all the patients were applied 5-7 ml/kg Ringer Laktat fluid replacement. All patients were preoxygenated with 10 L/dk %100 of oxygen for 1 minute. Anesthetic induction was done with 2.4 mg/kg of propofol and 1 µg/kg of fentanyl. Intubation was done 3 minutes after 0.6 mg/kg of rocuronium was given as a muscle relaxant agent. Following the intubation, a high current of 6L/dk was applied for 5 minutes. Maintenance of anesthesia was done with 1-2 MAC sevoflourane in group 1, keeping O_2 at 50% and N2O at 50% and with 1-2 MAC

sevoflourane in group 2 under 4 L/dk carrier gas, keeping O2 at 50% and air at 50% . Maintenance analgesia need was met in group 2 with 1 mcg/kg/saat fentanyl infusion. Ventilation tidal volume (TV) was kept at 6-8 ml/kg, I:E ratio at 1:2 and respiratory frequency was maintained in a way to keep Et CO2 at 35-40 mmHg, which makes normocapnia possible. FiO_2 value was preserved between 30 and 35%. Anesthetics were stopped and patient was ventilated with 100% of O_2 when the last skin suture was begun before the opration ended. Muscle relaxant was antagonised with 0,05 mg /kg of neostigmin and 0,01 mg/kg of atropine. The patient was respirated by hand after the antagonists were applied and spontaneous respiration was controlled every ten seconds. In order to determine the levels of TNF-α, IL6, IL10, blood samples were taken from all the patients at the 60th minute of anesthesia after anesthetic agents were stopped and at the post-operative 24th hour

Taking samples

After vascular access was obtained, blood samples were taken from all the patients before anesthesia (pre-operative), at the 60 the minute of anesthesia (intra-operative 60 min.) and after anesthetic agents were stopped(post-operative 0 min.). After the blood samples were congealed, they were centrifuged at 3500 rpm for 5 minutes. The serums separated after centrifuge were kept to be analysed at─80 C.

TNF-α measurtement

The measurement of TNF-α level was done using Serum TNF-α kit (Catalog No: KAP1751: 1 plate, Nivelles,BioSource firmasının TNF Belgium) which is based on solid phase sandwich ELISA principle.

IL-10 measurement

The measurement of Serum IL-10 level was done using IL-10 kit (Catalog No: KHC0101: 1 plate, Camarillo, USA) by Invitrogen which is based on solid phase sandwich ELISA principle.

IL-6 measurement

The measurement of Serum IL-6 level was done using IL-6 kit (Catalog No: KHC006: 1 plate, Camarillo, USA) by Invitrogen which is based on solid phase sandwich ELISA principle.

Statistical Analysis

Statistical evaluation was done using the SPSS 18.0 program. Descriptive statistics were expressed as mean±standard deviation for numeric data and as number and percentage for categorical data. Compatibility of the measured variables with normal distribution was examined with the Kolmogorov-Smirnov test. Significance test of the difference between two means was used for the differences of measured variables between the groups. Differences of categorical variables between the groups were evaluated with chi-square test. Changes in measured variables in time for the repetitive measurements were examined with single direction variance analysis. Paired comparisons were done with Bonferroni test when analysis yielded any differences. Results were evaluated in 95% cofidence interval and statistical significance was taken as $p<0.05$.

Results

In this study, blood samples taken from 22 ASA I-III patients (15 males and 7 females) who, receiving nitrous oxide, underwent elective urological surgical initiative that lasted 1 to 4

hours in the main operation room at our hospital and who were at an age interval of 17-49 (33.0±1.4) and the 22 patients (15 males and 7 females) who received no nitrous oxide and who were at an age interval of 21-46 (33.2±1.9) were examined.

No statistically significant difference was found between the groups in terms of sex and age (p=1.000, p=0.954). Defining characteristics regarding the sexes and ages of the patients are given in Table 1.

Table-1: Demographic characteristics of the patients

	Group 1		Group 2		p
Sex	n	%	n	%	1.000
Male	15	68.2	15	68.2	
Female	7	31.8	7	31.8	
Age (year)*	33.0±1.4 (21-46)		33.2±1.9 (17-49)		0.954

Group 1: The patients who received nitrous oxide at general anaesthesia
Group 2: The patients who received no nitrous oxide at general anaesthesia
* The results were indicated as average±SD (min-max) .

The measurement values of TNFα, IL-6, IL-10 belonging to the group of nitrous oxide-administered patients at preoperation, intraoperation 60th minute, postoperation 0th minute and postoperation 24th hours are given in Table 2.

Table-2: The significance of change in parameters depending on time in the nitrous oxide-administered patient group *

	Preop. 0th min	Intraop 60th min.	Postop 0th min.	Postop 24th h.	p
TNF-α (pg/ml)	61.56±58.53	37.56±46.02	9.66±5.87	7.97±4.26	<0.001
	(21.3-553.3)	(13.9-360.1)	(14.9-36.2)	(10.3-30.3)	
IL-6 (pg/ml)	17.07±21.03	13.15±26.25	20.30±30.97	36.00±38.95	<0.001
	(2.0-68.1)	(3.9-125.0)	(4.3-110.8)	(5.6-125.0)	
IL-10 (pg/ml)	3.76±3.08	4.19±2.75	3.89±2.88	4.74±3.62	0.100
	(1.0-15.9)	(1.4-13.4)	(1.9-15.0)	(1.9-17.7)	

* The results were indicated as average±SD (min-max) .

The change in TNFα depending on time in the nitrous oxide-administered patient group was found to be significant (p<0.001). While the statistical difference between TNFα levels at postoperation 0th min. and postoperation 24th hour was not significant, the difference between levels of TNFα was significant at all the other times. The change in TNFα averages depending on time is given in Figure 1.

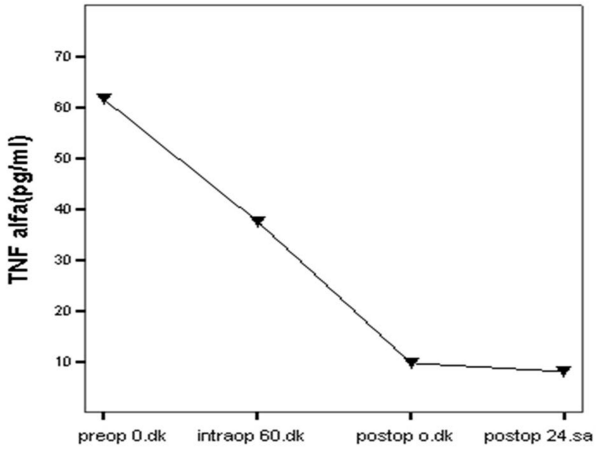

Figure-1: TNFα averages in the nitrous oxide-administered patient group depending on time

The change in IL-6 depending on time in the nitrous oxide-administered patient group was found to be significant ($p<0.001$). The difference between the levels of IL-6 at Postoperation 24th hour and those at all the other times was found to be significant. There was no significant difference between the levels of IL-6 at other times. The change in IL-6 averages depending on time is given in Figure 2.

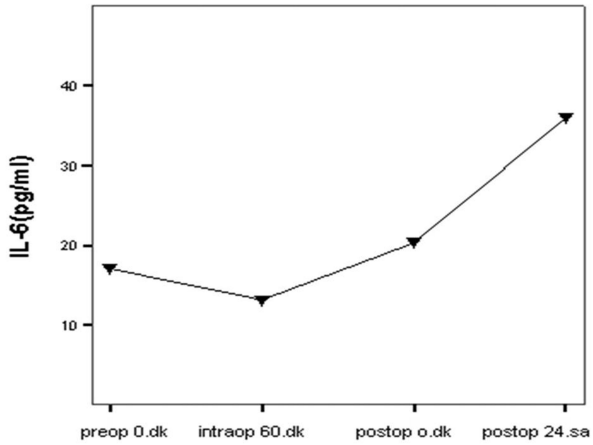

Figure-2: IL-6 averages in the nitrous oxide-administered patient group depending on time

No significant difference was found between times in terms of IL-10 in the nitrous oxide-administered patient group ($p=0.100$). The change in IL-10 averages depending on time is given in Figure 3.

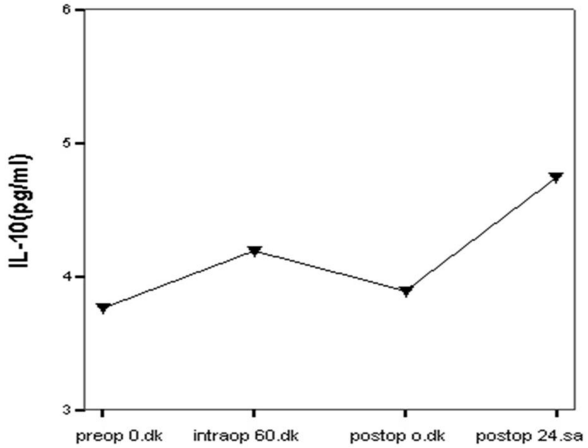

Figure-3: IL-10 averages in the nitrous oxide-administered patient group depending on time

The measurement values of TNFα, IL-6, IL-10 belonging to the group of patients who were not administered nitrous oxide at preoperation, intraoperation 60th min, postoperation 0th min. and postoperation 24th hours are given in Table 3.

Table-3: Significance of the change in parameters in the group of patients who were not administered nitrous oxide depending on time*

	Preop. 0th min.	Intraop. 60th min	Postop. 0th min.	Postop. 24th h.	p
TNF-α (pg/ml)	4.68±1.82	4.48±2.41	4.45±3.11	3.49±1.42	0.029
	(2.4-10.6)	(2.5-13.8)	(2.0-16.7)	(1.5-7.3)	
IL-6 (pg/ml)	3.00±0.81	2.52±0.23	2.99±0.56	3.59±3.63	0.008
	(2.0-4.3)	(2.0-3.1)	(2.3-4.2)	(2.3-19.8)	
IL-10 (pg/ml)	2.54±1.19	2.14±1.04	1.82±0.58	2.42±2.07	0.127
	(1.2-7.0)	(1.1-6.0)	(1.0-3.6)	(1.0-10.9)	

* The results were indicated as average±SD (min-max) .

The change in TNFα depending on time in the group of patients who did not receive nitrous oxide was found to be significant(p=0.029). The difference between TNFα values at postoperation 24th hour and the other times was found to be statistically significant. No statistically significant difference was found between the values at other times. The change in TNFα averages depending on time is given in Figure 4.

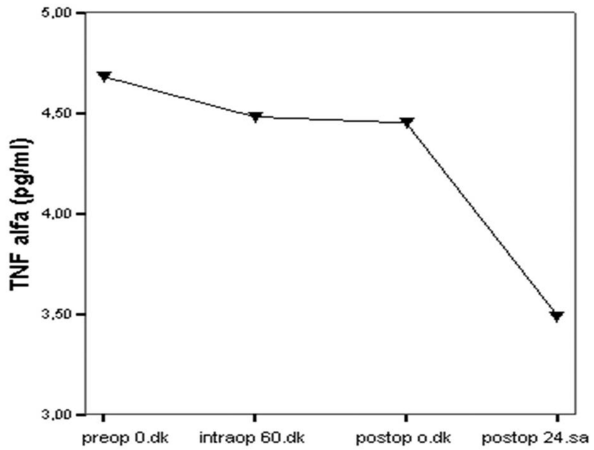

Figure-4: TNFα averages depending on time in the group of patients who did not receive nitrous oxide

The change in IL-6 depending on time in the group of patients who did not receive nitrous oxide was found to be significant (p=0.008). In the paired comparisons between preoperation and intraoperation 60th min., intraoperation 60th min. and postoperation 0th min, intraoperation 60th min. and postoperation 24th hour, the difference between IL-6 values was found to be statistically significant. No significant difference was found between IL-6 values at other times. The change in IL-6 averages depending on time is given in Figure 5.

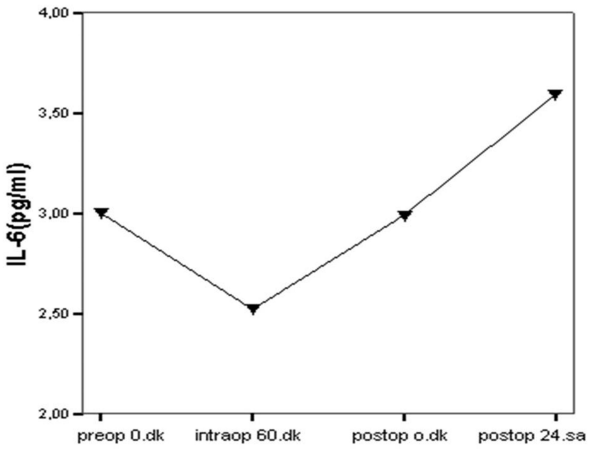

Figure-5: IL-6 averages depending on time in the group of patients who did not receive nitrous oxide

No significant difference between times in terms of IL-10 was found in the group of patients who did not receive nitrous oxide (p=0.127). The change in IL-10 averages depending on time is given in Figure 6.

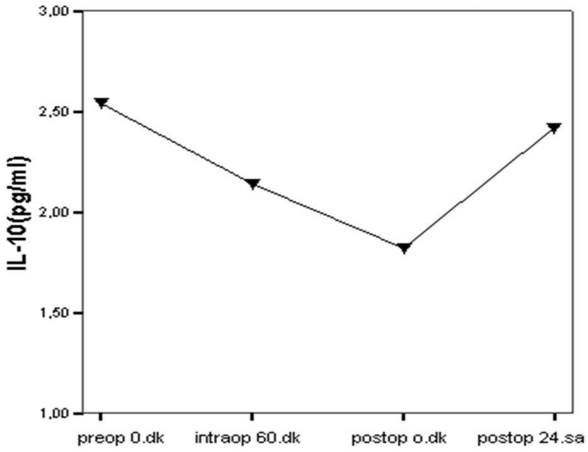

Figure-6: IL-10 averages depending on time in the group of patients who did not receive nitrous oxide

Discussion

Responses to a surgical trauma show variations with regard to metabolism, endocrine and immunity systems. These variations are related to the stimulation of afferent neurons in the surgery area, secretion of cytokines from the damaged tissue and activation of cellular and humoral immunity (15). Systematic inflammatory response, though it is a physiological and protective reaction associated with host defense, may lead to adverse changes in many organs by stimulating the overproduction of cytokines as a result of tissue damage (8).

Acute phase response trauma develop following a surgical initiative and infection and is characterized with the production of acute phase proteins (16). Inflammatory cytokines are known to play an important role in this response. Overproduction of cytokines causes the induction of adhesion molecules in leucocyte and endothelium cells and lecocyte sequestration and activation in lungs. These cytokine related reactions may result in systemic inflammatory response syndrome or multiple organ failure (17).

Nitrous oxide has commonly been used as an anaesthetic on humans since the nineteenth century. Nitrous oxide has a longstanding safety record and is generally considered to be a relatively safe anesthetic. Nitrous oxide is considered as a safe anaesthetic (18). Besides, it has an influence on the immunity system. In vivo and in vitro studies have revealed that nitrous oxide causes a decrease in the movement and chemotaxis of neutrophiles (14). Also, it has been determined that nitrous oxide affects the expression of endogenous sugar receptors on leukocytes and the cascade of leukocyte adhesion-activation after an anaesthesia which involves the usage of nitrous oxide (14, 19). However, it has such side effects as megaloblastic anemia and homocysteinemia. It also brings such risks as atherosclerosis, thrombosis, cognitive disfunction, neurotoxicity and probable teratogenicity. Increase intracranial pressure and cerebral blood flow and causing hypoxia, postoperative nausea and vomitting and probable immunosuppression are some of its known effects(18) . IL-6 is the most important cytokine that stimulates the synthesis of acute phase proteins in liver cells. It has such effects as differentiation, proliferation and activation of T cells, antibody production of B cells (20). Plasma level of IL-6 is also thought to be the indicator of tissue damage (21). The density of IL-6 is low in circulation and may not sometimes be determined. IL-6 begins to increase 30 to 60 minutes after surgical initiative has started and reaches high concentrations in 2 to 4 hours. This increase peaks at postoperation 24th hour and continues for approximately 72 hours (22). The half-life of IL-6 is approximately 1 hour (8).

Various studies into the effects of different anaesthetic techniques on cytokine levels have been done. In their study with 35 pregnants, Dermitzaki et al. gave general anaesthesia to 18 pregnants and neuroaxial anaesthesia to 17 pregnants and investigated the effects of neuroaxial anaesthesia as opposed to general anaesthesia on the levels of serum cytokine.

They found that serum IL-6 levels were significantly high 24 hours after caesarean section in both anaesthesia methods (23).

In a study which investigated the effects of different anaesthesia methods on cytokine response, it was revealed that IL-6 levels in circulation increased in a total hip replacement surgery postoperatively in both general and neuroaxial anaesthesia methods and this increase peaked 4 hours after the surgery (24). In another study which consisted of 20 cases of abdominal histerectomy, comparison of the nitrous-oxide administered group with the propofol and alfentanyl-administered group revealed that IL-6 levels in the nitrous-oxide administered group showed an earlier and higher increase (25). It was found IL-6 levels in the nitrous-oxide administered group to have increased during and after a major abdominal surgery. This increase showed a decrease on the first postopertion day but it is not significant statistically. In this study, the peak plasma IL-6 concentration which formed at the end of surgery was shown to be related to the loss of blood during surgery (26). In a study which investigated the effects of anaesthesia techniques on immune function and stress response in radical oesophagectomy patients, IL-6 levels were found to be significantly high in both the group that was given general anaesthesia with nitrous-oxide and the group that was given epidural anaesthesia on postoperation 1 and 3 days compared to the preoperation levels (27).In parallel with the other studies, our study, too, detected an increase in IL-6 levels in both groups. However, when postoperation and preoperation IL-6 levels are compared, the increase (211%) in the nitrous-oxide administered group is seen to be higher than that in the group which was not given nitrous-oxide (119%). This makes us think that nitrous-oxide activates the production of IL-6. Although our study does not handle the source of cytokines, increased IL-6 levels suggest that they were released from the damaged surgery area.

TNFα is an important inflammatory mediator. TNFα is responsible for a host of physiological changes such as hypotension, fever, tachycardia and oliguria. It also has antigenic activity and this activity causes secretion of prostaglandin, IL-6, IL-8 and tissue factor-III from monocytes endothelial cells (28). TNFα is a proinflammatory cytokine which increases blood-brain barrier permeability and induces the synthesis of endothel adhesion molecules by enabling inflammatory mediators to be released (29).

In a study which investigated the effects of general anaesthesia and regional anaesthesia on serum cytokine levels, while a decrease was determined in serum TNFα levels of patients in the preoperation and postoperation periods, it was not found to be significant (23). In another study which investigated the effects of general anaesthesia and spinal anaesthesia that involved the usage of nitrous oxide on stress response in hemorrhoidectomy patients, TNFα was observed to drop at postoperation 24th hour. However, this is not statistically significant (30). It was determined that usage of nitrous oxide in general anaesthesia in the rats which were subjected to brain ischemia reperfusion injury led to a statistically significant drop in TNFα levels (31). In a study which investigated the effects of epidural anaesthesia on immunosuppression during abdominal surgery, a significant drop in TNFα levels during surgery was observed in the group that was given general anaesthesia (32). In another study, a significant decrease in the production of TNF-α was seen two hours after upper abdominal surgery started and reached the minimum level at the end of the surgery (33). In our study, while no change was observed in TNFα levels during surgery in the general anaesthesia group which was not given nitrous oxide, a significant decrease was seen at postoperation 24th hour. The drop, on the other hand, in TNFα levels in the general anaesthesia group that was given nitrous-oxide begins at intraoperation 60th min. and lasts until postoperation 0th min.

However, a significant change is observed at postoperation 0th min. and postoperation 24th hour.

And this suggests that nitrous oxide has an impact on TNF-α, whose half-life is approximately 6 minutes, in the first 60 minutes and increases this inhibitor effect during surgery.

Surgical trauma and anaesthesia are related to complex disfunction of the immune system in which both proinflammatory and antiinflammatory response are activated. The balance that changes between proinflammatory and antiinflammatory cytokines is thought to represent the mechanisms that cause immune disfunction during posoperative period (34, 28). IL-10 is secreted mainly from monocyte and Th2 cells (10). IL-10 is an immunoregulatory cytokine that functions as an antiinflammatory by suppressing the synthesis of proinflammatory cytokines (35, 36). It has been reported to play a role in systemic response in infections and inflammations and be produced during sepsis (37). In a study which investigated the effects of epidural anaesthesia on surgical stress, Kawasaki et al. observed that IL-10 level in the group that was given general anaesthesia increased after the surgery started, peaked at the end of surgery, began to decrease after the surgery and dropped to preop levels on the fourth day. They put forward that this increase in IL-10 level is related to the suppression mechanisms of the immune system (32). In a study which dealt with the effects of general anaesthesia and epidural anaesthesia with nitrous oxide on plasma IL-10 levels in 10 patients who underwent abdominal surgery, they observed that IL-10 levels began to increase two hours after the surgery started and peaked at the fourth hour. They showed that IL-10 levels began to decrease in postoperation period and receded to preop levels (38). In the general anaesthesia without nitrous oxide, IL-10 levels were observed to increase prior to anaesthesia, at the end of the surgery and postoperation 24th hour (39). A significant increase was detected in IL-10

levels, which were checked preoperation and postoperation, of 18 adult patients who underwent major surgery. In this study in whose general anaesthesia nitrous oxide was not used, they pointed out that IL-10 increase in the early postoperation period was a protective mechanism to limit inflammation after surgical trauma (39). In a study on 16 elective partial gastrectomy patients who were given general anaesthesia with nitrous oxide, it was reported that IL-10 levels increased during surgery and decreased at postoperation 24 hour (40). In the study which assessed antiimflammatory profile after surgery, no statistically significant change was detected in IL-10 levels, which were checked before and during surgery and on postoperation 1, 2 and 4. days (41).

In our study, while IL-10 levels of the group that was not given nitrous oxide decreased at postoperation 0 and postoperation 24 hours compared to preop levels, an increase was seen in the nitrous-oxide administered group. However, the changes in both groups were not found statistically significant and this suggests that both of the anaesthesia methods do not cause a significant antiimflammatory change on IL-10.

In conclusion nitrous oxide showed significant effect on inflammatory and antiinflamatory parameters. Further studies are needed to understand to eveluate the effects of nitrous oxide. The study of anestesia is a broad topic, and our group intends to investigate further its mechanism of regulation.

Official approval, dated 15.01.2009 and numbered 2009/01/15, to the effect that the present study is in compliance with ethical principles was obtained from the Ethical Committee at Karaelmas University Application and Research Hospital.

References

1. Stevenson WG, Hall CS, Rudnick S, et al: The effect of anesthetic agents on the human immune response. Anesthesiology. 1990;72:542-552.

2. Ayala A, Wang P, Ba FZ, et al: Differential alterations in plasma IL-6 and TNF levels after trauma and hemorrhage. Am J Physiol. 1991;260:R167-R171.

3. Cruse PJE, Foord R. A five year prospective study of 23,649 surgical wounds. Archives of Surgery. 1973;107:206-209.

4. Brodsky JB. In: Eger EI II, ed., Toxicity of Nitrous Oxide in Nitrous Oxide/O2. New York: Elsevier Science, 1985; 259-262.

5. Sheeran P, Hall GM. Cytokines in anaesthesia. British Journal of Anaesthesia. 1997;78: 201-219.

6. Laffey JG, Boylan JF, Cheng DC The systemic inflammatory response to cardiac surgery: implications for the anesthesiologist. Anesthesiology. 2002;97(1):215-252

7. Munoz C, Carlet J, Fitting C, et al: Dysregulation of in vitro cytokine production by monocytes during sepsis. J Clin Invest. 1991;88:1747-1754.

8. Biffl WL, Moore EE, Moore FA, Peterson VM. Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation? Ann Surg. 1996;224:647-664.

9. Beutler B, Cerami A. Cachectin: More than a tumor necrosis factor. N Engl J Med. 1987; 316:379-385.

10. Firentino FD, Zlotnik A, Mosmann RT, et al: IL-10 inhibits cytokine production by activated macrophages. J Immunol. 1991;147:3815-3822.

11. Vieira P, de Waal Malefyt R, Dang NM, et al: Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: Homology to Epstein-Barr virus open reading frame BCRFI. Proc Natl Acad Sci U S A. 1991; 88:1172-1176.

12. Mennerick S, Jevtovic-Todorovic V, Todorovic SM, Shen W, Olney JW, Zorumski CF. Effect of nitrous oxide on excitatory and inhibitory synaptic transmission in hippocampal cultures. J. Neurosci. 1998;18:9716-9726.

13. Daniel EB, and Rosenberg M Nitrous Oxide and the Inhalation Anesthetics. Anesth Prog. 2008;55:124-131.

14. Frohlic D, Rothe G, Wittmann S, Schmitz G, Schmid P, Taeger K, Hobbhahn J. Nitrous oxide impairs the neutrophil oxidative response. Anesthesiology. 1998;88:1281-1290.

15. Hall GM, Desborough JP. Interleukin-6 and the metabolic response to surgery. Br J Anaesth. 1992;69:337338.

16. Douglas RG, Shaw JH. Metabolic response to sepsis and trauma. BrJ Surg.1989.76:115-22.

17. Dinarello CA, Gelfand JA, Wolff SM: Anticytokine strategies in the treatment of the systemic inflammatory response syndrome. JAMA. 1993;269:1829-35.

18. Lehmberg J, Waldner M, Baethmann A, Uhl E. Inflammatory response to nitrous oxide in the central nervous system.B rain R eserach. 2008;1246:88-95.

19. Bardosi L, Bardosi A, Gabius HJ. Changes of expression of endogenous sugar receptors by polymorphonuclear leukocytes after prolonged anaesthesia and surgery. Can. J. Anaesth. 1992;39:143-150.

20. Naito Y, Tamai S, Shingu K et al. Responses of plasma adrenocorticotropic hormone, cortisol, and cytokines during and after upper abdominal surgery. Anesthesiology. 1992;77: 426-431.

21. Rixen D, Siegel J H, Abu-Salih A, Bertolini M, Panagakos F, Espina N. Physiologic state severity classification as an indicator of posttrauma cytokine response. Shock 1995;4:27-38.

22. Desborough JP. The stress response to trauma and surgery. Br J Anaesth. 2000;85:109-117.

23. Dermitzaki E, Staikou C, Petropoulos G, Rizos D, Siafaka I, Fassoulaki A. A randomized study of maternal serum cytokine levels following cesarean section under general or neuraxial anesthesia. Int J Obstet Anesth. 2009;18:33-37.

24. Hogevold HE, Lyberg T, Kähler H, Haug E, Reikerås O. Changes in plasma IL-1beta, TNF-alpha and IL-6 after total hip replacement surgery in general or regional anaesthesia. Cytokine 2000;12:1156-1159.

25. Crozier TA, Muller JE, Quittkat D. Effect of Anaesthesia on the Cytokine Responses to Abdominal Surgery BJA. 1994;72:280-285.

26. Kato M, Suzuki H, Murakami M, Akama M, Matsukawa S, Hashimoto Y. Elevated plasma levels of interleukin-6, interleukin-8, and granulocyte colony-stimulating factor during and after major abdominal surgery. J Clin Anesth. 1997;9:293-298.

27. Yokoyama M, Itano Y, Katayama H, Morimatsu H, Takeda Y, Takahashi T, Nagano O, Morita K. The effects of continuous epidural anesthesia and analgesia on stress response and immune function in patients undergoing radical esophagectomy. Anesth Analg. 2005;101:1521-1527.

28. Helmy SAK, Wahlay MAM and Nawaway M. The effect of anaesthesia and surgery on plasma cytokine production. Anaesthesia. 1999;54:773-738.

29. Fantuzzi G, Di Santo E, Sacco S, et al. Role of the hypothalamicpituitary- adrenal axis in the regulation of TNF production in mice. J Immunol. 1995;155:3552-3555,

30. Buyukkocak U, Caglayan O, Daphan C, Aydinuraz K, Saygun O, Agalar F. Similar effects of general and spinal anaesthesia on perioperative stress response in patients undergoing haemorrhoidectomy. Mediators Inflamm. 2006;97257:1-5.

31. Nellgard B, Mackensen GB, Massey G, Pearlstein RD, Warner DS. The effects of anesthetics on stress responses to forebrain ischemia and reperfusion in the rat. Anesth Analg. 2000;91:145-151.

32. Kawasaki T, Ogata M, Kawasaki C, Okamoto K, and Sata T. Effects of epidural anaesthesia on surgical stress-induced immunosuppression during upper abdominal surgery. British Journal of Anaesthesia. 2007;98:196-203.

33. Kawasaki T, Ogata M, Kawasaki C, Tomihisa T, Okamoto K, Shigematsu A. Surgical stress induces endotoxin hyporesponsiveness and an early decrease of monocyte mCD14 and HLA-DR expression during surgery. Anesth Analg. 2001;92:1322-1326.

34. Atwell DM, Grichnik KP, Newman MF, Reves JG, McBride WT. Balance of proinflammatory and antiinflammatory cytokines at thoracic cancer operation. Ann Thorac Surg.1998;66:1145-1150.

35. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol. 1993;11:165-190.

36. Mosmann TR, Sad S. The expanding universe of T-cell subsets: TH1, TH2 and more. Immunol Today. 1996;17:138-146.

37. Marchant A, Deviere J, Byl B, De Groote D, Vincent JL, Goldman M: Interleukin-10 production during septicaemia. Lancet. 1994;343:707-708.

38. Kato M, Honda I, Suzuki H, Murakami M, Matsukawa S, Hashimoto Y. Interleukin-10 production during and after upper abdominal surgery J . Clin Anesth. 1998;10:184-188.

39. Delogu G, Antonucci A, Signore M, Marandola M, Tellan G, Ippoliti F. Plasma levels of IL-10 and nitric oxide under two different anaesthesia regimens. Eur J Anaesthesiol. 2005;22:462-466.

40. Ogata M, Okamoto K, Kohriyama K, Kawasaki T, Itoh H, Shigematsu A. Role of interleukin-10 on hyporesponsiveness of endotoxin during surgery. Crit Care Med. 2000. 28:3166-170.

41. Lukaszewicz AC, Faivre V, Villa F, Payen D. Anti-inflammatory profile of circulating immune cells after surgery for seizure. Minerva Anestesiol. 2010;76:477-484.