scientific reports

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Effects of spinal versus general anesthesia on serum oxidative stress markers and cytokine release after abdominal hysterectomy: a non-randomized trial

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Anesthetic techniques can significantly influence postoperative outcomes by modulating inflammatory cytokine release and oxidative stress (OS). This study aimed to compare serum levels of OS biomarkers and inflammatory cytokines in patients undergoing abdominal hysterectomy under spinal anesthesia (SA) or general anesthesia (GA). A single-blinded, non-randomized controlled trial was conducted on forty-two patients (American Society of Anesthesiologists (ASA) class I and II) undergoing abdominal hysterectomy. Two groups of patients were assigned: SA and GA. To measure the levels of inflammatory cytokines (tumor necrosis factor-alpha [TNF-α], interleukin-1 beta [IL-1β], and interleukin-6 [IL-6]), as well as anti-inflammatory cytokine (interleukin-10 [IL-10]) and oxidative stress (OS) biomarkers (superoxide dismutase [SOD], catalase [CAT], and malondialdehyde [MDA]), blood samples were collected both before anesthesia and 48 h after surgery. Postoperatively, the serum levels of MDA significantly increased, while enzyme activity of CAT decreased in the GA group compared to pre-anesthesia levels. The MDA concentration was significantly higher in the GA group compared to the SA group. Besides, the SOD activity was significantly lower in the GA group than in the SA group. TNF-α and IL-6 serum levels were raised in the GA group postoperatively compared to pre-anesthesia levels. Furthermore, the concentration of TNF-α and IL-6 was significantly higher in the GA group than in the SA group. There was a notable difference in the ratio of IL-6/IL-10 between the two groups. GA significantly increased inflammatory factor levels and decreased serum antioxidants after abdominal hysterectomy compared to SA. These results indicate a lower inflammatory response to SA than GA.

Keywords Spinal anesthesia, General anesthesia, Hysterectomy, Oxidative stress, Cytokines

The postoperative period is characterized by neuroendocrine, immune, and metabolic alterations triggered by tissue injury, anesthesia, surgical stress, psychological distress, and postoperative pain[1]. The innate immune system, which mediates the release of proinflammatory cytokines and activates circulating neutrophils, tissue macrophages, and monocytes, plays a central role in the inflammatory response following surgery[2]. Alterations in T-cell subsets, reduced lymphocyte proliferation, decreased total lymphocyte counts, and a shift toward a T-helper type 2 (Th2) cytokine profile are commonly implicated in the suppression of adaptive immune responses during the early postoperative period[3].

Systemic pro- and anti-inflammatory immune responses are regulated by cytokines such as interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α). These cytokines act as key mediators

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of inflammation and are closely associated with the degree of tissue damage induced by immune activation. While TNF-α and IL-6 are proinflammatory, their release is suppressed by anti-inflammatory cytokines such as IL-10. In response to tissue injury, cytokines are secreted not only by immune cells—including mast cells, macrophages, and lymphocytes—but also by non-immune cells such as fibroblasts and endothelial cells. Cytokines play vital roles in host defense, modulation of post-traumatic pain, and the wound-healing process. Under normal conditions, circulating cytokine levels are low or undetectable within the first 30 to 60 min after the onset of surgery. Peak concentrations are usually observed around 24 h after major procedures and may remain elevated for 48 to 72 h postoperatively[4,5].

Beyond surgical trauma, anesthesia itself can modulate immune responses and potentially reduce perioperative complications by suppressing proinflammatory cytokine expression[2]. However, studies have reported inconsistent findings regarding the impact of various anesthetic agents[6–8] and techniques[9,10] on postoperative immune function and cytokine release. Oxidative stress (OS), a key component of the surgical stress response, is triggered in patients undergoing both spinal anesthesia (SA) and general anesthesia (GA), serving as an early contributor to organ damage[11]. OS results from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense systems. A tightly regulated balance of ROS and antioxidants is critical for maintaining normal cellular function[12]. Anesthetic stress can increase plasma cortisol levels, which in turn promote tissue injury through enhanced ROS production[11]. Moreover, different anesthetic agents may either exacerbate or alleviate OS, depending on their pharmacologic properties[12].

Hysterectomy, the surgical removal of the uterus, can be performed via abdominal or vaginal approaches, using either laparoscopic or open techniques. Although laparoscopic hysterectomy is less invasive, the open abdominal method remains more widely used due to certain technical limitations. As the second most common gynecological surgery, hysterectomy is frequently indicated for conditions such as chronic pelvic pain, inflammatory diseases, endometriosis, uterine prolapse, fibroids, abnormal uterine bleeding, and malignancy[13,14]. Each year, approximately 100,000 hysterectomies are performed in the United Kingdom (UK) and 600,000 in the United States[15,16]. This procedure can be conducted under either GA or SA, both of which offer distinct advantages. GA is often associated with greater patient comfort and increased convenience for the surgical team during lengthy procedures.

In contrast, SA offers several advantages, including improved hemodynamic stability and reduced intraoperative bleeding[17–19]. Regional anesthesia has been shown to decrease blood loss, lower the need for transfusions, and reduce the risk of postoperative complications during hysterectomy, thereby contributing to better surgical outcomes and higher patient satisfaction[18]. Moreover, patients receiving SA often experience shorter recovery times and improved postoperative recovery quality[20]. Nonetheless, each anesthetic approach is associated with specific risks. GA may lead to complications such as aspiration pneumonia, nausea, vomiting, and the potential need for postoperative mechanical ventilation[21]. SA can be associated with adverse effects including hypotension, post-dural puncture headache, and, in rare cases, neurological injury[22].

Few studies have concurrently assessed OS indicators and cytokine responses in the context of a normal surgical procedure despite growing interest in the immunomodulatory effects of anesthetic procedures. In order to reduce confounding factors associated with surgical complexity, comorbidities, or anesthetic heterogeneity, this study presents a novel and integrated method by analyzing these biomarkers in a homogenous surgical group undergoing abdominal hysterectomy. This thorough design improves internal validity and offers fresh perspectives on the ways in which anesthetic type affects OS and systemic inflammation. Therefore, this study aimed to compare the effects of spinal and general anesthesia on postoperative levels of pro- and anti-inflammatory cytokines and oxidative stress biomarkers in a homogeneous surgical population. W hypothesized that spinal anesthesia would lead to lower inflammatory response and oxidative stress compared to general anesthesia. The primary outcomes included TNF-α, IL-6, IL-1β, MDA, SOD, and CAT. The secondary outcomes were IL-10 levels and IL-6/IL-10 ratio.

Materials and methods

Study design and patient allocation

A single-blinded, non-randomized, controlled study was conducted at Afzalipour Hospital in Kerman, Iran, involving women patients scheduled for hysterectomy who were classified as American Society of Anesthesiologists (ASA) class I and II by the American Society of Anesthesiologists. Patients were excluded if they had a history of inflammatory or autoimmune diseases, endocrine disorders, diabetes mellitus, hypertension, or severe neurological conditions. Additional exclusion criteria included corticosteroid use within three months before surgery and any evidence of active or recent infectious disease. Furthermore, patients who experienced intraoperative complications—such as excessive bleeding requiring blood transfusion or the need for vasopressor support—were also excluded from the study.

Participant assignment was conducted after receiving approval from the Ethical Committee at Kerman University of Medical Sciences (IR.KMU.AH.REC.1401.154) and obtaining written consent from the patients. Women patients were assigned to two distinct study arms defined in this trial: Spinal anesthesia (SA) ($n=21$) or General anesthesia (GA) ($n=21$) (Fig. 1). The allocation was non-randomized and based on medical considerations and the patients' preferences whenever clinically feasible. The same surgical team performed all procedures and standardized postoperative care protocols. The study was single-blinded; laboratory personnel and data analysts were blinded to group assignments.

All methods conducted in the research were performed in accordance with the relevant guidelines of the Ethical Committee at Kerman University of Medical Sciences. This trial was registered at irct.behdasht.gov.ir/ with reference no. IRCT20190819044559N4 on 31/01/2023.

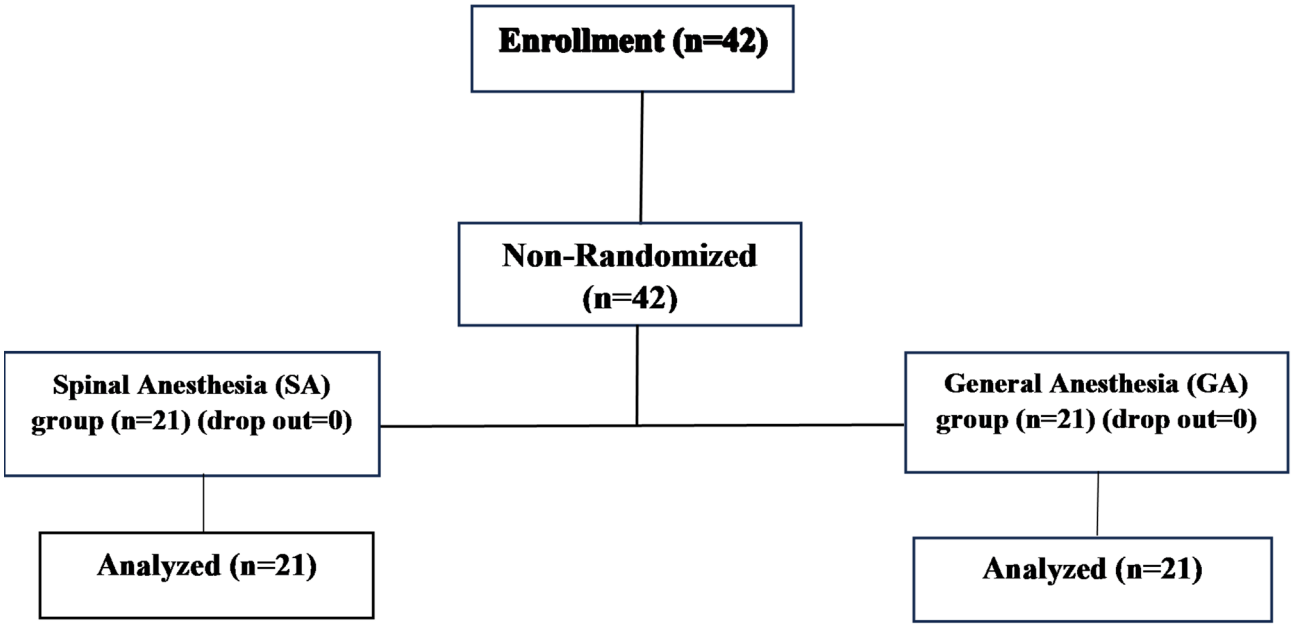


Fig. 1. The CONSORT Flow Diagram.

Anesthesia induction

All patients fasted for 10 to 12 h and received premedication with fentanyl (1.5 µg/kg) and midazolam (0.03 mg/kg) orally one hour before entering the operating room. Upon arrival in the operating room, the patients were monitored using a non-invasive automated blood pressure device, a pulse oximeter, and a three-lead electrocardiogram (ECG). A fluid preload of 400 to 500 mL of an electrolyte solution was administered through an 18-gauge intravenous cannula over 10 to 15 min before the initiation of eitherGA or SA.

GA was induced by fentanyl (1.5-3 macro/kg) and midazolam (0.03–0.5 mg/kg) as premedication, and for anesthesia, lidocaine (1 mg/kg), propofol (1.5-2 mg/kg), and Atracurium (0.5 mg/kg). Anesthesia was maintained with isoflurane (1.2 mac isoflurane) and oxygen/Nitrous oxide (N2O) mixture (3lit/min/3lit/min). We optimized the controlled mechanical ventilation (Primus, Dräger) to maintain normocapnia and guarantee an oxyhemoglobin saturation exceeding 95%.

SA was administered under strict sterile conditions following standard preparation and draping. A 25-gauge spinal needle was used to puncture the subarachnoid space between the fourth and fifth lumbar vertebrae. During the procedure, 20 µg of fentanyl, 20 mg of bupivacaine, and 10 µg of epinephrine were injected intrathecally.

Patients were continuously monitored for heart rate (HR), systolic and diastolic blood pressure (BP), mean arterial pressure (MAP), and peripheral oxygen saturation (SpO^2). Demographic characteristics of the patients, including addiction history, age, height, weight, BMI, and surgery duration, were also recorded. Blood samples were collected prior to anesthesia and 48 h post-operation. The samples underwent centrifugation, and the resulting supernatants were utilized for biochemical assays.

The standard 4-2-1 rule (i.e., 4 ml/kg/h for the first 10 kg, 2 ml/kg/h for the second 10 kg, and 1 ml/kg/h for each additional kg of body weight crystalloid fluid (normal saline or ringer)) was applied consistently across both anesthesia groups for intraoperative fluid administration. This standardised approach aimed to reduce fluid management variability and its possible effects on indicators of oxidative stress. Crystalloid fluids were given at a 3:1 ratio to the amount of blood lost during surgery to compensate for the loss. The study did not include patients who needed blood transfusions. Vasopressor support was not given to any of the study patients. The fluid management protocol was the same for all groups, and the estimated intraoperative blood loss was between 400 and 600 cc.

Study outcomes

The primary outcomes of the study were the changes in serum levels of pro- inflammatory cytokines (TNF-α, IL-6, interleukin-1 beta (IL-1β)) and oxidative stress markers (malondialdehyde [MDA], superoxide dismutase [SOD], and catalase [CAT]) before anesthesia and 48 h postoperative. The secondary outcomes included anti-inflammatory cytokine IL-10 and IL-6/IL-10 ratio changes, indicating the balance between pro- and anti-inflammatory responses.

Biochemical assays

MDA, a lipid peroxidation marker, was estimated in serum using a modification of the prior protocol involving trichloroacetic acid (TCA) and thiobarbituric acid (TBA). Absorbance was measured with a spectrophotometer at 532 nm (nm) and expressed as MDA (nmol/ml)[23]. The total activity of SOD was measured utilizing a Randox assay kit (London, England) based on the manufacturer's instructions. The outcomes were recorded based on U/

mg protein. To estimate the activity of the CAT enzyme, a specific volume of serum was mixed with potassium phosphate buffer and hydrogen peroxide. Then, the absorption rate was assessed to be 240 nm every 15 s. The reduction in absorption rate per minute was calculated. One unit of CAT activity is defined as the amount of the enzyme that breaks down one millimole of hydrogen peroxide per minute (KU/L)[24].

The serum concentrations of IL-1β and TNF-α, IL-6 and IL-10 were estimated using an enzyme-linked immunosorbent assay (ELISA) kit (CN: KPG-HIL1β, KPG-HTNF-α, KPG-HIL6, and KPG-HIL-10, respectively, Pars Gene Karmania Kit, Iran) following the manufacturer's instructions. The assignments for the study groups were kept confidential from the data analysts and laboratory personnel.

Statistical analysis

Sample size

Sample size calculation was performed based on data from previous studies [25,26], using a two-sided hypothesis test with a significance level of 0.05 and a statistical power of 90%. The effect size (Δ) was assumed to be 4.7 with a standard deviation (σ) of 4.2. The required sample size was estimated using the following formula[27]: $2n = 4(Z\alpha + Z\beta)2 \times \sigma 2$.

Where:

- • $Z\alpha = 1.96$ (corresponding to a two-sided test with $\alpha = 0.05$).
- • $Z\beta = 1.28$ (corresponding to 90% power).
- • $\sigma = 4.2$, $\Delta = 4.7$.

Substituting the values:

$2n = 4(1.96 + 1.282)2 \times (4.2)2 \approx 34$.

Thus, the minimum required sample size was calculated as 17 patients per group. To account for an estimated 20% dropout rate, the final sample size was increased to 21 patients per group.

Quantitative data was displayed as mean ± SD (standard deviation) and qualitative data as frequency (percentage). The Kolmogorov-Smirnov test was employed to assess the normal distribution of the data. Based on the obtained results, further analysis utilized a paired t-test for the parametric data and Mann-Whitney U for non-parametric data. Also, quantitative variables were used for analysis using one-way ANOVA (Analysis of Variance) and repeated measures. The qualitative variables were analyzed utilizing the chi-square test or Fisher's exact test. Data analysis was performed with SPSS version 22.0, supplied by SPSS Inc. in Chicago, IL, USA.

Results

Forty-two candidates for hysterectomy surgery, aged between 19 and 79 years, entered the study. The demographic and underlying characteristics of the patients are shown in Table 1. No significant difference was observed in the mean age of patients between the GA (46.71 ± 10.34) and SA (49.30 ± 10.57) groups ($P = 0.43$).

Variable	GA	SA	*P*-value
Age, years	46.7 ± 10.34	49.3 ± 10.57	0.86
Height	163.29 ± 9.7	160.36 ± 6.57	0.29
Weight	78.05 ± 12.42	65 ± 10.86	0.88
BMI	29.44 ± 4.77	25.28 ± 4.08	0.81
Surgery duration	112.05 ± 32.06	107.1 ± 27.3	0.56
Addiction **Yes** **No**	 1(4.8) 20(95.2)	 1(5) 19(95)	0.74
Hysterectomy cause Benign Malignant	 17(81) 4(19)	 13(65) 7(35)	0.3
SBP[a] 1 h before anesthesia During surgery End of surgery Recovery	 146.09 ± 27.18 131.72 ± 14.56 141.09 ± 19.07 136.27 ± 15.78	 131.22 ± 35.12 19.41 ± 108.33 110.66 ± 13.95 114.94 ± 12.27	0.47
MAP 11 h before anesthesia During surgery End of surgery Recovery	 105.16 ± 19.54 99.9 ± 11.42 100.16 ± 12.34 101.36 ± 11.86	 100.98 ± 15.73 80.72 ± 15.92 83.56 ± 12.28 88.29 ± 9.28	0.16
HR[a] 1 h before anesthesia During surgery End of surgery Recovery	 81 ± 14.35 73.18 ± 12.78 76.36 ± 14.35 76.81 ± 14.45	 84.11 ± 11.57 73.88 ± 10.84 75.33 ± 9.1 76.72 ± 10.24	0.43

Table 1. Demographic data, blood pressure and heart rate of patients with general and spinal anesthesia. Data are displayed as mean ± SD or frequency (percentage). BP: Blood pressure; HR; Heart rate; SA: Spinal anesthesia; GA: General anesthesia. SBP: Systolic blood pressure; MAP: Mean arterial pressure; [a] the analysis was used for BP, and HR repeated measures to compare the two groups at different times.

Parameter		SA	*p*-Value		GA	*p*-Value
	Pre	post		Pre	Post	
MDA(nmol/mL)	1.27 ± 0.09	1.02 ± 0.12	< 0.001	0.92 ± 0.19	1.2 ± 0.2*	< 0.001
CAT (KU/L)	64.6 ± 4.9	69.15 ± 4.55	< 0.001	69.05 ± 5.49	61.95 ± 4.55*	< 0.001
SOD (U/L)	46.77 ± 8	48.88 ± 8.52	0.88	55.87 ± 8.34	57.14 ± 7.32	0.88

Table 2. Effect of anesthesia on serum levels or enzyme activities of oxidative stress markers. Data are displayed as mean ± SD. $^{*}P < 0.05$ vs. pre-anesthesia level utilizing paired t-test. SA: Spinal anesthesia; GA: General anesthesia; SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde.

Cytokines	GA	SA	*P*-Value
MDA (nmol/mL)*	1.24 ± 0.09	1.06 ± 0.2	0.046
CAT(KU/L)	65.5 ± 4.95	66.92 ± 6.15	0.26
SOD (U/L) *	56.79 ± 8.05	63.1 ± 30.5	0.001

Table 3. Comparison of changes in serum levels or enzyme activities of oxidative stress markers between the SA and GA groups. Data are displayed as mean ± SD. $^{*}P < 0.05$ vs. between two group utilizing one-way ANOVA. SA: Spinal anesthesia; GA: General anesthesia; SOD: superoxide dismutase; MDA: malondialdehyde; CAT: catalase.

Also, no significant difference was observed in the addiction variables and reasons for hysterectomy between the study groups ($p > 0.05$) (Table 1). The SBP of people undergoing hysterectomy with GA lowered at the end of the surgery ($P = 0.48$), during recovery ($P = 0.09$) and one hour after the surgery ($P = 0.28$) compared to the SBP before the surgery, which is not statistically significant. However, in the SA group, the SBP at the end of the surgery ($P = 0.01$), during recovery ($P = 0.02$), and one hour after the surgery ($P = 0.04$) had a statistically significant reduction in comparison with the SBP before the surgery. The research groups did not show any statistically significant differences in how their SBP and MAP changed over time (Table 1) ($P = 0.47$ and $P = 0.16$, respectively). Additionally, the reduction of HR in the GA group during recovery and the end of the surgery was insignificant. The HR at the surgery end ($P = 0.004$), during the recovery period ($P = 0.004$), and after surgery ($P = 0.002$) was statistically significantly lower in the SA group than it was before anesthesia. Besides, HR changes between the study groups at different times were insignificant ($P = 0.34$) (Table 1).

The impact of anesthesia methods on serum oxidative stress factor levels

Table 2 displays the serum enzyme activities of the antioxidant factors SOD and CAT, as well as the oxidant level of MDA, in the two groups prior to anesthesia and after surgery. The analysis demonstrated no statistically significant differences ($P < 0.05$) in the serum enzyme activities of SOD, CAT, and level of MDA between the two groups before anesthesia. Following surgery, the GA group's serum MDA level was considerably higher than before anesthesia ($P < 0.001$). Moreover, the CAT enzyme activity decreased significantly after the surgery compared to the time before anesthesia ($P < 0.001$); however, SOD changes were not significant ($P = 0.88$). In the SA group, although the CAT and SOD enzyme activities increased after the surgery compared to before anesthesia; however, the changes were not statistically significant. Besides, no significant change in MDA levels was observed in the SA group after surgery compared to before anesthesia (Table 2).

The serum level of CAT in the SA group was not significantly higher than in the GA group ($P = 0.26$). The SA group had significantly higher SOD enzyme activity than the GA group ($P = 0.001$). Additionally, the GA group's serum MDA level was significantly higher than that of the SA group ($P = 0.046$) (Table 3).

The impact of anesthesia methods on the serum anti-inflammatory and pro-inflammatory factor concentrations

Prior to anesthesia, the two groups (SA and GA) did not significantly differ in their serum levels of the cytokines TNF-α, IL-6, IL-10, and IL-1β. The GA group's post-operative TNF-α and IL-6 levels were significantly greater than their pre-anesthesia values ($P = 0.001$ and $P = 0.002$, respectively). Following surgery, the GA group's IL-1β cytokine level diminished relative to its pre-anesthesia level; however, the reduction was not statistically significant ($P = 0.11$). Regarding IL-10, while its level rose post-surgery in the GA group, this increase did not reach statistical significance ($P = 0.8$) (Table 4).

While all anti-inflammatory and inflammatory variables, such as IL-10 ($P = 0.45$), IL-6 ($P = 0.69$), and TNFα ($P = 0.90$), were higher in the SA group following surgery than prior to anesthesia, this difference was not statistically significant. Furthermore, alterations in IL-1β serum levels post-surgery showed no significant difference in the SA group ($P = 0.45$) (Table 4).

The variations in inflammatory factor serum concentrations between the two groups are shown in Table 5. The SA group exhibited significantly lower levels of all inflammatory variables assessed than the GA group. However, no significant difference was observed in the serum levels of IL-10 between the two groups.

Parameter		GA	*p*-Value		SA	*p*-Value
	Pre	Post		Pre	post	
IL-6 (pg/ml)	44.33 ± 13.96	69.58 ± 29.86*	0.002	30.46[20.39–34.31]	74.94 ± 46.86	0.69
IL-1β(pg/ml)	50.53 ± 15.02	41.1 ± 13.89	0.11	46.86 ± 15.65	41.13 ± 19.87	
TNF-α(pg/ml)	22.18[19.2-32.67]	33.98 ± 24.09*	0.001	22.99 ± 8.1	23.55 ± 7.6	0.9
IL-10(pg/ml)	9.48[8.9-11.98]	12.92 ± 7.68	0.8	9.44 ± 3.69	11.51 ± 4.33	0.45

Table 4. Effect of anesthesia on Anti-inflammatory and Pro-inflammatory factors serum levels. Data are displayed as mean ± SD. *$P < 0.05$ vs. pre-anesthesia level using paired t-test. GA: General anesthesia; SA: Spinal anesthesia; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-10: Interleukin-10; TNF-α: Tumor necrosis factor-alpha.

Cytokines	GA	SA	*P*-Value
IL-6 (pg/ml)*	63.77 ± 45.5	53.88 ± 52.63	< 0.001
IL-1β (pg/ml)*	46.56 ± 7.78	44.26 ± 17.57	0.009
TNF-α (pg/ml)*	60.08[38.13–68.72]	23.79 ± 7.9	< 0.001
IL-10 (pg/ml)	22.29[17.23–27.5]	10.63 ± 4.05	0.06

Table 5. Alterations comparison in serum cytokine levels between the SA and GA groups. Data are displayed as mean ± SD. *$P < 0.05$ one-way ANOVA. SA: Spinal anesthesia; GA: General anesthesia; IL-6: Interleukin-6; IL-1β: Interleukin-1 beta; IL-10: Interleukin-10; TNF-α: Tumor necrosis factor-alpha.

Cytokine ratio	GA	SA	*p*-value
IL-6:IL-10*	3.5 ± 7.2	3.2 ± 5.8	0.028
TNF-α:IL-6	0.48 ± 1.3	0.42 ± 0.8	0.066

Table 6. Comparison of changes in ratios of serum cytokine levels in SA and GA group. Data are displayed as mean ± standard deviation. *$P < 0.05$ one-way ANOVA. SA: Spinal anesthesia; GA: General anesthesia; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1 beta; IL-10: Interleukin-10.

Table 6 illustrates the ratio of serum cytokine levels in the SA and GA groups. The SA group's TNF/IL-6 inflammatory factor ratio was lower than that of the GA group; however, the difference was insignificant ($P = 0.06$). In contrast, the IL-6/IL-10 ratio in the SA group was statistically lower than in the GA group ($P = 0.028$).

Discussion

Given the significance and implications of surgery, selecting an appropriate anesthesia method is crucial for minimizing the stress response during the procedure and reducing immune damage in the body. Thus, this study investigates the effects of general and spinal anesthesia on the changes in serum levels of pro-inflammatory and anti-inflammatory cytokines, as well as OS factors, following hysterectomy. The study's results demonstrated that when scrutinizing the level of OS factors, the level of MDA as an oxidant factor in the GA group displayed a significant increase after surgery compared to before anesthesia. Also, the enzyme activity of CAT as an antioxidant factor decreased significantly after surgery compared to the time before the anesthesia. The serum levels of OS factors in the SA group did not show a significant difference after surgery compared to before the anesthesia. In addition, SOD enzyme activity was higher in the SA group than in the GA group, while MDA levels were higher in the GA group compared to the SA group. The IL-6 and TNF-α levels in the GA group showed a significant increase after surgery compared to the time before the anesthesia; however, no significant difference was observed in the level of IL-1β. In the SA group, the level of IL-1β, IL-10, IL-6, and TNFα cytokines had no significant difference before anesthesia and after surgery. The serum levels of inflammatory factors were lower in the SA group compared to the GA group, and this difference was statistically significant in all cases except for IL-10. Furthermore, the SA group's ratio of the inflammatory factor IL-6 to the anti-inflammatory factor IL-10 was lower than that of the GA group.

OS plays a significant role in the surgical stress response for patients undergoing SA and GA, and it is a primary factor in causing organ damage[11]. Stress caused by anesthesia increases cortisol, an endogenous stress marker that causes tissue damage by releasing pro-inflammatory cytokines and ROS[3,28]. Generally speaking, there are two methods of ROS production, which include non-electron transport (non-ER), electron transport (ER) pathways, and redox cycling. The first pathway involves ethers, ethanol, and haloalkanes, and the next pathway includes fentanyl, propofol, ketamine, and ranitidine[29]. Diverse non-enzymatic and enzymatic antioxidants help sustain normal physiological levels of ROS, highlighting the significance of biomedical antioxidants[30,31]. Therefore, different biomarkers have been created to assess OS level[29]. Numerous studies have examined the impact of different anesthetics on OS markers; however, the findings may vary due to differences in patients'

health statuses, and the types of surgeries performed[32]. Our research shows a significant increase in serum MDA levels in patients with GA following surgery compared to levels measured before anesthesia. Additionally, we observed a notable decrease in catalase activity in these patients after surgery compared to measurements taken prior to anesthesia. This is indicative of the increased OS. However, there was no significant difference in the serum levels of MDA, enzyme activities of CAT, and SOD before and after surgery in patients with SA. Additionally, MDA and SOD showed a significant difference between the two groups, which demonstrated a reduction in OS in SA patients compared to GA. Prior studies have demonstrated that anesthesia increases OS, which depends on the type of drugs used during the surgery and the depth of anesthesia[33,34]. So far, few studies have evaluated OS factors in patients undergoing various surgeries with different anesthesia methods. Along with the results of our study, significant changes in catalase activity and MDA concentration were observed in patients undergoing ORIF surgery with GA after the surgery compared to the time before the surgery. Accordingly, the MDA concentration, a marker of lipid peroxidation, and catalase activity showed significant changes in patients with GA after surgery compared to before surgery, which indicates an increase in OS after general anesthesia[35].

There is a tight connection between the nervous, endocrine, and immune systems. The intricate interactions among hormones, particularly adrenal corticosteroids, cytokines, and acute phase reactants, modify immune defense mechanisms in response to surgical stress[36]. Surgical stress activates biochemical pathways that release pro-inflammatory cytokines, leading to increased side effects, impaired wound healing, and delayed recovery after surgery. Additionally, long-term inflammation due to impaired innate immunity or ongoing pathology from cellular stress may serve as a prognostic factor for elevated ROS production and chronic diseases[29,34]. Inflammation has emerged as a significant focus in relation to cellular and mechanical stress, especially during surgical procedures. OS is believed to be essential in the body's response to chronic inflammatory conditions by aiding the migration of immune cells to sites of damage or inflammation[37]. It has been demonstrated to improve the conversion of several inflammatory mediators and proteins, such as nuclear factor-kappa B (NF-ΚB), which subsequently releases inflammatory cytokines[29,34]. So, this process activates various cytokines, inflammatory enzymes, and C-reactive proteins in response to external stimuli, such as oxidative stress. Furthermore, the interaction between OS and inflammation is significant for patients exposed to various anesthetic agents[30,34]. Surgical stress triggers the IL-6 and TNF-α release, crucial for initiating and sustaining inflammatory processes during surgery, activated by the Hypothalamic-Pituitary-Adrenal (HPA) axis[3,28,38]. Consistent with the current study's findings, Liu et al. (2019) showed that patients with gastric cancer undergoing tumor resection under general anesthesia had significantly higher levels of inflammatory factors like high-sensitivity C-reactive protein (hs-CRP), IL-1, IL-8, and TNF-α than patients undergoing surgery under a combination of GA and epidural anesthesia[25]. Zura et al. reported that the level of the pro-inflammatory cytokine IL-6 was elevated in both the SA and GA groups. However, IL-2 levels were higher in the GA group compared to the SA group[3]. In another study, Heijmans evaluated the effects of four different anesthetic techniques on the inflammatory response during cardiac surgery with cardiopulmonary bypass and found that interleukin-6 levels were significantly lower in patients who received thoracic epidural anesthesia compared to those who underwent general anesthesia[39]. The study by Wu et al. demonstrated that inflammatory markers were significantly lower in patients who received a combination of spinal and general anesthesia compared to those who received GA alone during laparoscopic hernia surgery[40]. Furthermore, a clinical trial conducted by Vosoughian et al. reported that serum levels of TNF-α and IL-6 were significantly higher after cesarean section compared to preoperative levels in the GA group. GA significantly increased TNF-α and IL-6 levels following cesarean section (C-section) compared to spinal anesthesia. GA significantly increased the levels of TNF-α and IL-6 following C-section compared to SA. Additionally, in the aforementioned study, the level of the anti-inflammatory cytokine IL-10 did not differ significantly between the GA and SA groups, which is consistent with the findings of the present study[6]. IL-10 is an anti-inflammatory cytokine that may regulate the production of pro-inflammatory cytokines[6]. One study reported that plasma levels of IL-10 increase during the perioperative period and return to baseline levels within a few days after surgery[41]. In this context, a study conducted by Arma in 2020 aimed to compare the effects of SA and GA anesthesia on OS and inflammatory cytokines in orthopedic surgery. The results showed no significant difference in IL-6 levels between the two groups. However, patients in the SA group exhibited higher levels of TNF-α after surgery[35]. Epidural and general anesthesia can influence inflammatory responses either by directly affecting immune cell functions—such as those of lymphocytes, monocytes, and neutrophils—or by modulating the pain and stress responses following surgery[1,42]. Moreover, anesthesia management may alter IL-6 production after surgery. Regional anesthesia has been shown to have minimal impact on IL-6 levels following hip surgery[43]. However, both epidural and general anesthesia have demonstrated significant but temporary effects on various immune parameters. These include neutrophil respiratory burst activity, T lymphocyte subset ratios, neutrophil phagocytic activity, natural killer (NK) cell activity, and plasma levels of a wide range of pro- and anti-inflammatory cytokines, such as TNF-α, IL-1α, IL-2, IL-6, IL-1β, IL-10, IL-1Ra, IL-12, Transforming growth factor beta (TGF-β), and Interferon (IFN)[42]. These findings underscore the complex interplay between anesthesia techniques and immune function, highlighting the need for further investigation. The effects of anesthesia management on postoperative immune responses vary. Studies examining the impact of epidural anesthesia on postoperative immune function have reported inconsistent results. Some have shown that epidural anesthesia preserves NK cell activity and reduces the surgical stress response in patients undergoing hysterectomy[44]. Notably, epidural anesthesia has also been associated with significant reductions in norepinephrine, epinephrine, cortisol levels, NK cell ratios, and alterations in lymphocyte subset distribution, regardless of pain levels[45]. Additionally, patients undergoing abdominal surgery who received controlled epidural analgesia exhibited reduced pro-inflammatory cytokine responses (IL-6, IL-2, and IL-1β) and lower lymphocyte mitogenic proliferation compared to those receiving patient-controlled analgesia[45].

Local anesthetics not only block neuronal transmission but also act as potent anti-inflammatory agents[1]. According to the findings, the addition of certain adjunct medications to spinal or general anesthesia may

contribute to reducing the levels of inflammatory mediators. Furthermore, variations in inflammatory marker levels among patients in different groups may be attributed to differences in surgical procedures, underlying medical conditions, and individual biological variability. The timing of serum sampling for inflammatory and oxidative stress markers following surgery and anesthesia also plays a critical role and may account for discrepancies observed across different studies.

This study has several limitations. First, inflammatory marker levels were not measured at multiple time points, such as during the perioperative period, 4 h postoperatively, and 24 h after surgery. As a result, temporal comparisons between the two groups were not possible. Second, the study included only one type of surgical procedure—abdominal hysterectomy. While this was an intentional decision to maintain homogeneity in surgical stress, anesthesia protocol, and perioperative management (thereby improving internal validity), it may limit the generalizability of the findings. Future studies should include a variety of surgical procedures, including minimally invasive (e.g., laparoscopic or vaginal hysterectomy) and non-gynecological surgeries, to validate and expand upon these results. Finally, the non-randomized design of this study limits the ability to draw causal inferences. Randomized controlled trials are recommended in future research to confirm these findings and strengthen the existing evidence base.

Consequently, patients who underwent general anesthesia GA exhibited higher levels of inflammatory markers and significantly lower serum antioxidant levels than those who received SA. These findings suggest that SA induces a milder inflammatory response in the body than GA. Overall, surgery is a significant physiological stressor that can impact immune function and inflammatory regulation. Employing an appropriate anesthesia technique that minimizes tissue injury, inflammation, and stress responses may help reduce postoperative complications and improve patient outcomes.

Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Received: 4 February 2025; Accepted: 13 August 2025
Published online: 18 August 2025

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Author contributions

M.H. conception or design of the work, conduction of anesthesia and approved final version of manuscript, S.S.K collected samples, Z.H. performed surgery, Z.T. performed surgery, H.T. conduction of anesthesia, F.D. analysis data, L.A. Conception or design of the work, interpretation of data, and writing and editing original draft.

Declarations

Competing interests

The authors declare no competing interests.

Ethics

The objectives of the study and the confidentiality of personal information were explained to the participants before requesting their completion of the informed consent form. After obtaining the code of ethics from the Ethics Committee of Kerma University of Medical Sciences (IR.KMU.AH.REC.1401.154), the eligible candidates entered the study. This trial was registered at irct.behdasht.gov.ir/ with reference no. IRCT20190819044559N4 on 31/01/2023.

Additional information

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