Spinal Anesthesia and Minimal Tissue Trauma Improve the Outcome of Elective Cesarean Section

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Abstract

Background: Immune balance is mandatory for proper postoperative (PO) courses and wound healing. Cesarean section is the commonest surgical procedure for females. The choice of anesthetic procedure may affect the mother and fetal outcomes.

Objectives: Evaluation of the impact of general (GA) versus spinal anesthesia (SA) on parturient' cytokines' serum levels.

Patients and methods: 73 and 59 parturients received GA and SA, respectively. Fetal APGAR scoring was determined at 1-min & 5-min PO. PO pain severity was evaluated using the numeric rating scale and the duration of analgesia was calculated. Blood samples (S1, S2, S3) were obtained for ELISA estimation of serum interleukins and tumor necrosis factor-α (TNF-α). The study outcome is the effect of the anesthetic procedure on serum cytokines levels.

Results: Cytokines' levels were significantly higher in S2 and S3 than in S1 samples of all parturients with significantly higher levels in samples of GA patients. Percentages of change in serum cytokines' levels were higher with GA than with SA. Receiver operating characteristic (ROC) curve defined serum levels of TNF-α as the most cytokine affected by the anesthetic procedure. APGAR scores were significantly higher at 1-min and the duration of PO analgesia was significantly longer with SA.

Conclusion: SA can lessen the surgery-induced release of inflammatory cytokines, while GA augments this effect. Moreover, neonatal and maternal outcomes were superior with SA than with GA.

Keywords: General anesthesia; Spinal anesthesia; Cesarean section; Inflammatory cytokines; Surgical stress.

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Introduction

The recent WHO guidance in 2021 statement is a cesarean section (CS) is essential only to improve maternal and perinatal outcomes and prevent or reduce mortality and morbidity for the fetus and mother, which may reach up to 10-15%.

The applied anesthetic procedure may modulate the surgical stress by increasing or decreasing oxidative stress and release of inflammatory cytokines according to the applied procedure **(Aremu et al.,2020)**. Tissue injury per se can activate phagocytes to produce leukotriene B4, which increases the production of chemokines and pro-inflammatory cytokines **(Salina et al.,2020)**. Moreover, skin injury provokes the secretion of cytokines and growth factors necessary for local immuno-protection and tissue repair **(Kanno et al.,2017)**.

Tumor necrosis factor (TNF)-α is a potent pro-inflammatory cytokine that plays a major role in the initiation of the activation cascade of other cytokines and growth factors **(Tanabe et al.,2010)**. Interleukins (IL) are pleiotropic cytokines that may act as inflammatory, anti-inflammatory, or both actions and are secreted by a variety of cells **(Brocker et al.,2010)**. Interleukin-6 (IL-6) and IL-1β play a major role in the acute phase response, IL-6 is characterized by contradictory functions according to the pathophysiological base for its increased release **(Fietta et al.,2014)**, while IL-1β plays an active role in the pathogenesis of multiple pathological conditions through the activation of inflammasome **(Liu et al.,2016)**.

Spinal anesthesia (SA) for CS is nowadays a popular plan of anesthesia because of its rapid onset and high frequency of successful blockade **(Demilew et al.,2019)**. However, maternal hypotension is its most frequent complication **(Alemayehu et al.,2020)**. The study aimed to assess the impact of the type of anesthesia on surgical stress as manifested by the serum levels of inflammatory cytokines in parturients undergoing CS.

Patients and methods

Design: Comparative clinical trial

Setting: Departments of Anesthesia, Pain and ICU and Medical Biochemistry, Faculty of Medicine, Benha University.

Inclusion criteria: Pregnant women free of exclusion criteria and had singleton fetuses and an indication for CS and signed written informed consent were enrolled in the study.

Exclusion criteria: Premature rupture of membranes, preterm labor, fetal distress necessitating rapid intervention, gestational hypertensive disorders, gestational or overt diabetes, presence of acute inflammatory reaction manifestations, maintenance of immunosuppressive drugs, cancer, renal and cardiac disorders, or autoimmune disorders.

Ethical considerations: The preliminary approval of the study protocol by the Benha University Ethical Committee was obtained in Oct 2020. All parturients assigned for elective CS were clinically evaluated by obstetricians for timing and indication of CS, inclusion and exclusion criteria. The study protocol was discussed in full detail with each parturient fulfilling the inclusion criteria and those accepted to participate in the study signed the written consent. The final approval was obtained after the end of case collection in May 2022 by RC: 2.5.2022.

Sample size: A previous comparative study on the impacts of general versus neuraxial anesthesia for CS on plasma cytokines' levels included 35 parturients divided into two groups and reported non-significant differences between both anesthetic techniques **(Dermitzaki et al.,2009)**. This non-significant difference could be attributed to the small sample size; thus, the current study was supposed to have a significant difference when the sample size per group was >55 parturient and if so, the study power will be 85% with an α value of 0.05 and β value of 0.15

Grouping: Parturients who fulfilled the inclusion criteria (n=132) were allowed to choose between receiving general or spinal anesthesia according to their preference after explanations of the pros & cons of each procedure. Seventy-three parturients preferred general anesthesia (GA group) and fifty-nine parturients preferred to receive spinal anesthesia (SA group) with comparable enrolment data to that of patients enrolled in group GA (**Table 1, Fig. 1**).

Table 1. Parturient data

Data	Group GA (n=73)	Group SA (n=59)
Age (years)	26.8±3.7	27±2.9
Weight (kg)	87±4.4	86.2±5
Height (cm)	169±2	168.7±1.5
Body mass index (kg/m^2)	30.5±1.7	30.3±1.8
Gestational age (weeks)	38.8±1.2	39±0.8
Operative time (min)	40.9±6.9	39.1±7.1

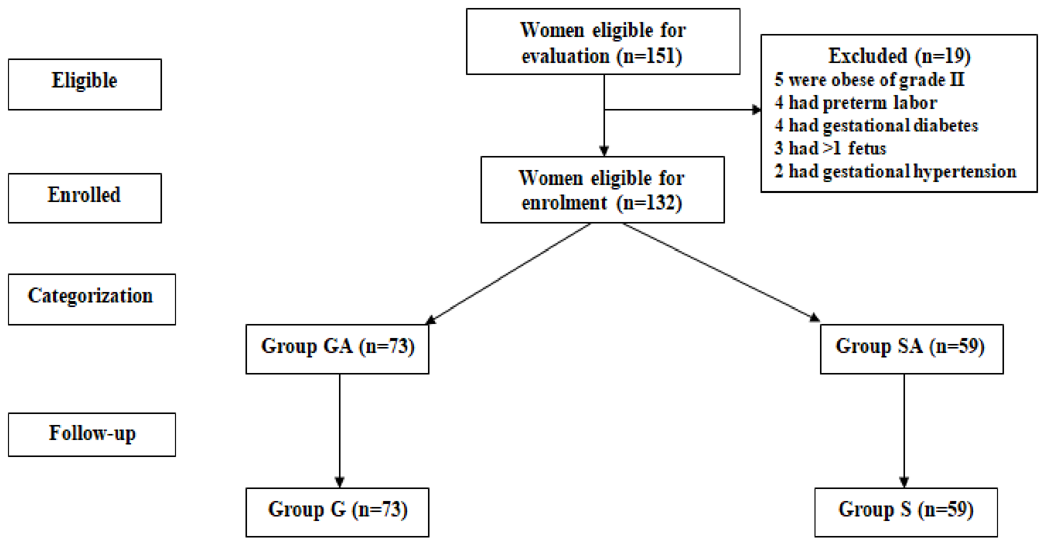

Fig. (1): Flow chart of the study

Methods

Anesthetic techniques: All parturients were monitored non-invasively for heart rate (HR), mean arterial pressure (MAP), oxygen saturation (Sp_{O2}), and fetal heart rate.

1. **General anesthesia (GA)**
   All GA parturients were pre-oxygenated for 3-5 min with 100% O_2. Propofol (2 mg/kg) was used for induction of anesthesia and rocuronium (0.6 mg/kg) was injected intravenously to facilitate tracheal intubation using cuffed 6.5-mm endotracheal tube. Anesthesia was maintained with isoflurane 1.2 MAC, top-up doses of rocuronium if needed and

fentanyl (1-2 μg/kg) was given after fetal delivery. During anesthesia tidal volume was set at 8 ml/kg and 12 breaths/min respiratory rates. After skin closure, neostigmine (0.05 mg/kg) and atropine sulfate (0.01 mg/kg) was given intravenously as a reversal for muscle relaxation.

2. **Spinal anesthesia**

Patients of the SA group received preload with 500 ml of lactated Ringer's solution. With the parturient in the sitting position, a 25-gauge spinal needle was inserted at the level of L_{4-5} or L_{3-4} and 12.5 mg of bupivacaine 0.5% was injected intrathecally. Then, the parturient was turned to a supine position.

Intraoperative and PO monitoring

- During the operative time, hypothermia was prevented by the use of pre-warmed fluids, adjustment of room temperature, and the use of a warming blanket if necessary. MAP was continuously monitored to guard against the drop in blood pressure; hypotension was defined as a decreased MAP by >20% of preoperative pressure and was treated with the rapid infusion of lactated Ringer’s solution and intravenous boluses of ephedrine. Cephalosporin; 3rd generation was given as prophylaxis after induction of anesthesia and was continued postoperatively to guard against the development of infection. Non-steroid anti-inflammatory drugs were prohibited for their suppressing effect on inflammatory mediators.

PO evaluation

1. **The fetal outcome** was evaluated using the APGAR scoring system at 1-min & 5-min after delivery on a 10-point scale **(Boyle.,1993)**.
2. **The intensity of PO pain** was assessed using a 0-10 point numeric rating scale (NRS) **(Fairbank et al.,1980)**, at the time of PACU transfer and every 30-min throughout 4-hr PO. Duration of analgesia was calculated from theater discharge till the 1st request of PO analgesia that was provided as a pethidine (0.5-1 mg/kg) injection and several requests for PO analgesia were also recorded.

Laboratory Investigations

Three blood samples (S1-3) were collected before induction of anesthesia, at end of the surgery, and 24-hr postoperative (PO) for estimation of serum levels of serum IL-1β, IL-6, and TNF-α using ELISA kits (Abcam Inc., San Francisco, USA, catalog no. ab46052, ab187013, and ab46087, respectively) and were read using a 96 well microplate ELISA reader (Dynatech. MR 7000):

The study outcome is the change in serum cytokines' levels in S2 and S3 samples in comparison S1 samples according to the applied anesthetic procedure.

Statistical analysis

The obtained results were analyzed by SPSS for Windows statistical package (Version 22, 2015; IBM, Armonk, USA) using the One-way ANOVA test, paired t-test, and Chi-square test (X^2 test). The ROC curve analysis was performed to determine which cytokine could be seriously affected by the applied anesthetic procedure. P value <0.05 was considered statistically significant.

Results

Operative time was non-significantly longer in the GA group. APGAR score at 1-min was significantly (P=0.012) higher in the SA group, but the 5-min APGAR score was significantly higher than the 1-min score in both groups with a non-significant inter-group difference. Five neonates required admission to NICU; 4 in group GA, but only one in group SA with a non-significant difference in favor of SA group SA. Thirty-five (59.3%) parturients in the SA group did not require PO analgesia till the time of discharge, and 24 parturient (40.7%) required it once. On contrary, 54 parturients (74%) in the GA group required PO analgesia two times, and 19 parturients (26%) required it once with a significantly (P<0.001) higher difference between both groups. Duration of PO analgesia was significantly (P<0.001) longer in patients of the SA group compared to patients of the GA group (**Table 2**).

Table 2. PO maternal and neonatal data of both groups

Data		Group GA	Group SA
Operative time (min)		40.1±7.3	38.2±6.2
APGAR score	**1-min**	8.3±1.5	8.9±1.3*
	5-min	9.5±0.8†	9.7±0.6†
Number of requests of PO analgesia	**0**	0	35 (59.3%) ‡
	1	19 (26%)	24 (40.7%)
	2	54 (74%)	0
Postoperative duration of analgesia (min)		71.5±23.8	230±14.4‡

* and ‡: indicates a significant difference between both groups at P<0.05 and <0.001, respectively; ‡: indicates a significant difference between 1-min and 5-min APGAR score at p<0.001

Pain scores till 90-min PO were zero for all patients of the SA group and then increased gradually for patients who requested PO analgesia. On the other hand, in patients of the GA group pain increased gradually since the end of surgery and peaked at 60-min PO whenever all patients received PO analgesia and plateaued at NRS score of two till 210 min, and patients who requested the second injection showed decreased pain score (**Fig. 2**).

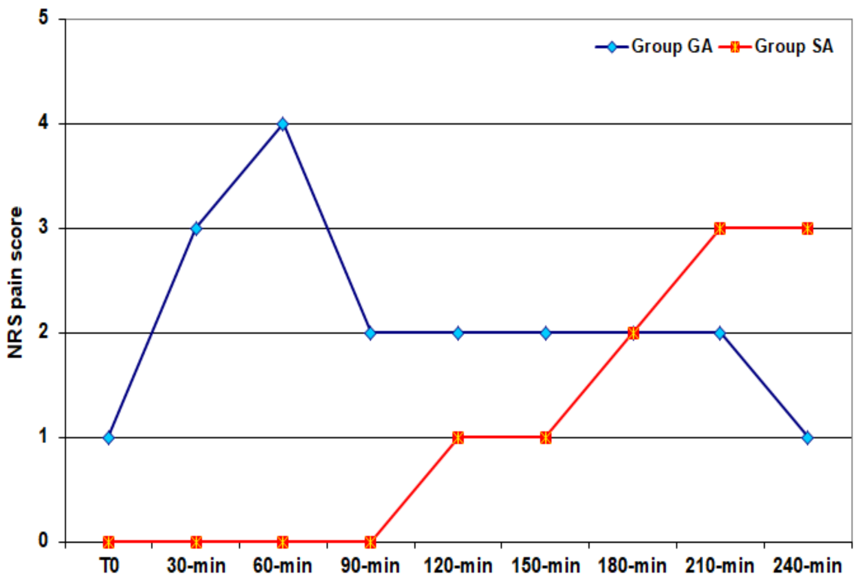

Fig. 2: Postoperative NRS pain score of parturient of both groups

Estimated serum cytokines' levels in S1 samples showed non-significant (P=0.828) differences between patients of both groups. However, estimated levels of the three cytokines increased significantly in S2 and S3 samples compared to levels estimated in the S1 sample with significantly higher levels in S3 samples than in S2 samples of all patients.

Estimated serum levels of TNF-α were significantly (P<0.001) higher in S2 than S1 samples of all patients with significantly (P<0.001) lower ΔTNF-α (S2-S1%) in patients of SA than in the GA group. Levels of TNF-α estimated in the S3 sample were increased significantly than S1 levels and were significantly higher in S3 samples than S2 samples of SA patients, but were decreased significantly in S3 than S2 samples of GA patients. However, estimated serum levels of TNF-α were still significantly (P=0.001) higher in S3 samples of GA than in SA patients, but the ΔTNF-α (S3-S2%) was significantly better in favor of GA. On contrary, serum levels of IL-6 were progressively increased in each sample than the preceding one in all patients, but with non-significantly lower in S2 (P=0.316) and S3 (P=0.872) samples of patients of SA than in GA groups. The calculated ΔIL-6 (S2-S1%) was significantly (P<0.001) lower, while ΔIL-6 (S3-S2%) was significantly (P=0.024) higher in samples of patients of SA than samples of patients of GA groups. Serum levels of IL-1β were also increased progressively in samples of all patients but were significantly (P<0.001) lower in S2 and S3 samples of SA than GA patients with significantly (P<0.001) lower ΔIL-1β (S2-S1%), but non-significantly (P=0.758) lower ΔIL-1β (S3-S2%) samples of SA than GA patients (**Table 3**).

Table 3. Mean levels of studied cytokines estimated in parturient of both groups

Parameters		**Group GA**	**Group SA**
TNF-α (ng/ml)	**S1**	4±0.51	4.2±0.68
	S2	6.56±1.1†	5±0.76†*
	ΔTNF-α (S2-S1%)	37.6±12.1	16±5*
	S3	5.9±1†‡	5.4±0.7†‡*
	ΔTNF-α (S3-S2%)	↓7.73±7.1	↑7.5±3.9*
IL-6 (ng/ml)	**S1**	8.66±2.64	9.3±3.81
	S2	14.7±4.5†	13.3±4.85†
	ΔIL-6 (S2-S1)	40.3±8.4	30.7±8.7*
	S3	17.83±4.57†‡	17.3±5.2†‡
	ΔIL-6 (S3-S2)	18.45±7.4	24.4±6.1*
IL-1β (ng/ml)	**S1**	4.63±1.66	4.43±1.63
	S2	5.72±1.56†	5±1.7*
	ΔIL-1β (S2-S1)	20.7±10.7	11.7±9.4*
	S3	6.17±1.57†	5.34±1.73†‡*
	ΔIL-1β (S3-S2)	7.73±3.59	6.86±3.2

*: Significant difference between both groups; †: Significant difference versus S1 sample**;** ‡: Significant difference versus S2 sample

ROC curve analysis showed that serum levels of the studied cytokines are seriously affected by the type of anesthesia as shown in figure 3. However, TNF-α levels are the most affected by the type of anesthesia with significantly higher AUC difference compared to AUC for IL-6 and IL-1β, but the AUC difference between IL-6 and IL-1β was non-significant (**Table 4 and Fig.3)**

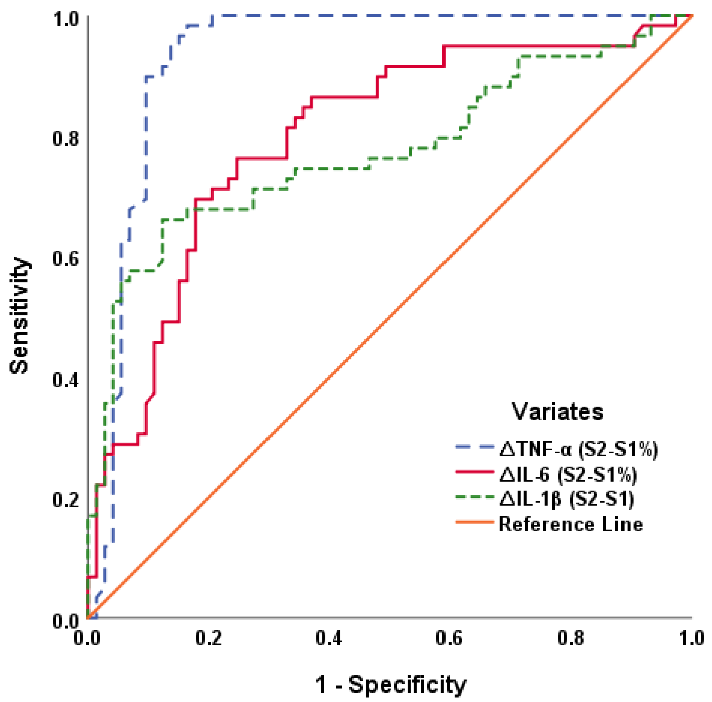

Fig. 3. ROC curve analysis for the relation between type of anesthesia and percentage of change (S2-S1) in serum inflammatory cytokines' levels

Table 4. ROC curve analysis for the relation between type of anesthesia and change of serum inflammatory cytokines' levels

ROC curve analysis				
Variables	AUC	SE	P	95% CI
TNF-α	0.933	0.025	<0.001	0.884-0.982
IL-6	0.789	0.040	<0.001	0.721-0.876
IL-1β	0.774	0.043	<0.001	0.689-0.859
Paired-sample area difference under the ROC Curves				
	AUC difference	SE difference	P	95% CI
TNF-α vs. IL-6	-0.135	0.255	0.006	[-0.231]-[-0.038]
TNF-α vs. IL-1β	-0.159	-0.261	0.001	[-0.256]-[-0.061]
IL-6 vs. IL-1β	-0.024	0.287	0.647	[-0.128]-[-0.080]

Discussion

The detected higher levels in samples of patients GA group than in samples of patients in the SA group illustrated the effect of the type of anesthesia on immune balance and run in line with the results of previous comparative studies between GA and SA (**Aremu etal.,2020; Jafarzadeh et al.,2020; Ganjifard et al.,2021; Vosoughian et al.,2021**).

The pathogenesis of GA-associated higher levels of inflammatory cytokines is still a matter of debate; one study accused opioids of triggering changes in cytokine release in the direction of inflammation after detecting perioperative reduction of serum levels of IL-12 and TNF-α for 48-hr after opioid-free anesthesia than after opioid-based GA (**Titon et al.,2021**). Another study accused surgery per se depending on the detection of increased expression of IL-6 mRNA levels before the end of the surgery, but these expression levels were significantly decreased at 48 h after major surgery under opioid-based GA and continued postoperatively on opioid analgesia, this study attributed this to the significant reduction of DNA methylation secondary to decreased levels of levels DNA methyltransferase-1 and 3 as a response to surgical stress rather than to opiate exposure **(Caputi et al.,2021).**

The reported significant differences between GA and SA regarding the percentages of change in serum cytokines' levels in successive samples indicated the beneficial effect of SA for control of the release of pro-inflammatory cytokines and for minimizing the immune milieu deregulation. Similarly, previous studies documented that combined spinal/general anesthesia **(Wada et al.,2007)** or SA alone **(Koksoy et al.,2013)** could preserve the T helper 1/T helper 2 balances more than GA alone.

In support of the suppressive effect of SA on cytokines' release, the reported increase of serum IL-6 levels at 24-hr PO after SA, while levels were decreased after GA, despite being still higher than after SA. Following this finding, recent studies reported increased serum IL-6 during and after surgery under GA, while only after surgery in patients received adjuvant blocks (**Matas et al.,2021; Bloc et al.,2021**).

The detected higher serum levels of inflammatory cytokines at the end of surgery manifested the effect of surgery on patients' immune milieu and support the results of the earlier animal models that reported increased levels of TNF-α at 30 min after wounding, peaked at 1-hr after injury, the rebound of levels till 48 h after wounding, and levels were inclined thereafter **(Wang and Ding.,2003)** and the in-vitro studies that detected time-dependent increased expression of IL-1β mRNA **(Bai et al.,2008)**, IL-6 and TNF-α mRNA (**Takamiya et al.,2009)** using real-time fluorescent quantitative PCR.

These findings regarding the effect of surgical trauma on serum cytokines' levels could be attributed to the multiplicity of cells of origin of these cytokines, by epidermal keratinocytes, dermal fibroblasts, and macrophages (**Zubair et al.,2012**), myoblasts (**Pillon et al.,2013**), or through the initial infiltration of macrophages in the muscle tissue (**Tominaga et al.,2019**). The pleiotropic nature of these cytokines could be another explanation, where in vitro studies detected a regulatory role of the sensor component of the inflammasome, on IL-1β production, which stimulates the production of keratinocyte-derived chemokine that in association with macrophage inflammatory protein 1α lead to the release of other inflammatory cytokines within the wound area and surrounding tissues (**Hu et al.,2010**).

Conclusion

Surgical stress is a strongly induced deregulated immune balance in the inflammatory direction. Spinal anesthesia can lessen the release of inflammatory cytokines, while GA augments the effect of surgical stress resulting in significantly higher serum levels compared to the use of SA. Moreover, neonatal and maternal outcomes were superior to SA.

Limitation

Estimation of serum anti-inflammatory cytokines' levels would help to evaluate the extent of the disturbance in an immune milieu in parturient undergoing CS under general or spinal anesthesia.

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