Effect of Different Anesthetic Techniques on Cytokine Gene Expression in Patients who Underwent Elective Cesarean Section

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ABSTRACT

Cesarean section (CS) is an important challenge for a pregnant woman and her newborn. The most common anesthesia techniques used for CS are general anesthesia (GA) and spinal anesthesia (SA). This study was designed to compare the modulation of genes whose expression level is indicative of the immune system following exposure to GA and SA.

The present study was performed on 40 women who were scheduled for elective CS receiving GA or SA. The expression levels of the relative mRNA of Interleukin (IL)-4, IL-6, IL-10, IL-17, Interferon (IFN)-γ, and tumor growth factor (TGF)-β before anesthesia (T0) and 24 hours post-anesthesia (T1) were analyzed by real-time polymerase chain reaction (RT-PCR) technique.

Twenty-four hours post-anesthesia, the expression levels of IL-10, IL-17, and IFN-γ genes were decreased while the expressions of IL-4, IL-6, and TGF-β genes were upregulated in two groups, however, the differences were not significant. The mRNA level of IL-4 was increased in the SA group significantly.

The post-CS mRNA levels of IL-4 in the SA group may indicate that SA is more appropriate than GA for the initiation of tissue repair pathways.

Keywords: Cytokines; Gene expression; General anesthesia; Spinal anesthesia

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INTRODUCTION

Cesarean section (CS) is one of the most common and the oldest surgeries that is performed on women.[1]

General anesthesia (GA) was the selective method for CS for many years. This method has some advantages, including rapid induction, cardiovascular, and respiratory stability. Nonetheless, GA agents may cause neonatal depression. In addition, some complications, including maternal aspiration and failed tracheal intubation have been reported.[2] The tendency towards spinal anesthesia (SA) has increased to avoid maternal and neonatal complications.[3] However, the condition of the newborn can be influenced by hypotension and uterine-peritoneal perfusion damage caused by the sympathetic block after SA.[4]

Anesthesia affects the activity of immune cells and the secretion of cytokines. There is a constant balance between pro-inflammatory and anti-inflammatory cytokines. Interleukin 6 (IL-6) and interferon-gamma (IFN-γ) are the important pro-inflammatory cytokines. IL-10 and IL-4 are the most important anti-inflammatory cytokines.[5] IL-17 directs some inflammatory factors against microorganisms in immune system-related diseases.[6] Transforming growth factor-beta (TGF-β) has multiple activities such as cell differentiation, inhibition of cell growth to modulation, and suppression of immune and inflammatory responses.[7]

Anesthesia affects the activity and number of immune cells. The effects of anesthesia on B-lymphocytes, T-lymphocytes, NK cells, macrophages, and leukocytes have been studied.[8] Anesthesia affects the secretion of cytokines and the biological behavior of some cells and suppresses the transcription of several genes.[9,10] Lowes et al studied brief isoflurane anesthesia effect on gene expression in rat brain. They reported that isoflurane affects differential gene expression.[11] Pan et al and Sakamoto et al showed that isoflurane alters several genes such as genes involved in drug metabolism.[10,12] Dang et al reported that spinal anesthesia or general anesthesia combined with epidural anesthesia is better than general anesthesia in reducing surgery-related immune inhibition.[13] Some scholars believe that local anesthesia cannot protect the immune function.[14,15]

Considering that CS is an important challenge for the life of a pregnant woman and her newborn, and disagreement about which anesthesia technique is best for maintaining the balance of the immune system and the health of mother and baby, the present study was designed to compare the modulation of gene expression of IL-4, IL-6, IL-10, IL-17, IFN-γ and TGF-β following exposure to GA and SA.

MATERIALS AND METHODS

Study Population

This study was conducted on 40 women referred to the maternity hospital of Rafsanjan for elective CS. Inclusion criteria were the reading and writing ability, age range 20-40 years, singleton pregnancy, term, and uncomplicated pregnancy. Exclusion criteria included a history of hypertension, diabetes, cardiovascular disease, immunodeficiency, and hepatitis B, mental disorders, inflammatory diseases, medication during pregnancy (except medications routinely used during pregnancy). The ethics committee of Rafsanjan University of Medical Sciences approved this study (REC number: IR.RUMS.REC.1395.125).

The sample size was estimated to be 15 people in each group based on a significance level of 5%, a statistical power of 0.95. Further, μ1 was 0.17 in the GA, μ2 was 0.05 in the SA and σ was 0.1 in two groups. [16]

After entering the women to the operating room, the researcher explained the research method to the patients. If there was no contraindication for SA or GA, after providing informed consent, the patients randomly (using 20 sealed envelopes with the mark of G=general anesthesia and 20 with the mark of S=spinal anesthesia) were allocated to one of the groups. Subsequently, the patient's demographic and clinical characteristics, such as age, weight, and gestational age were recorded. To match the study conditions an expert gynecologist performed all of the CSs and only one expert anesthesiologist anesthetized all of the patients.

SA was performed in the sitting position using a 25G spinal needle (Quincke Spinal Needle, Japan) and 2.5 mL hyperbaric Marcaine 0.5% (AstraZeneca, Sweden) in the subarachnoid space between L3-L4 interspace.[17] Then the patient was placed in the Trendelenburg position and the sensory block level T4-T6 was induced.

GA was induced by intravenous sodium thiopental (4-6 mg/kg) followed by succinylcholine (1-1.5 mg/kg) to facilitate tracheal intubation. After tracheal intubation, GA was maintained using a 50% nitrous oxide/oxygen mixture and atracurium (0.2-0.3 mg/kg). After clamping the umbilical cord, fentanyl (1-2 μg/kg) was injected.

Blood Samples

Whole blood samples (1 mL venous blood) were obtained from all patients at two-time points: on the operation day (T0), and 24 hours after surgery (T1).[18,19] Blood samples were immediately put into an anticoagulant tube (1 mL blood +1.5 mg EDTA) to minimize storage-associated changes. All samples were kept cool (at refrigerated temperatures) during storage and transfer to the laboratory. In this study, the person who performed the laboratory tests was unaware of the grouping of patients.

RNA Extraction and cDNA Synthesis

Total RNA (5 μL) was extracted using TriZOL solution from 500 μL of peripheral blood by Pars Tous Kit (Pars Tous, Iran) and following the manufacturer's instructions. The NanoDrop device (DeNovix, USA) was applied to determine the purity and quantification of mRNA. Immediately after extraction, the cDNA was generated using the Easy cDNA Synthesis Kit (Pars Tous, Iran) in a final volume of 25 μL at 70°C for 10 minutes, 42°C for 60 minutes, and 96°C for 5 minutes (Thermocycler BIORAD, C1000). All cDNAs were kept at -20°C until the next testing process. The expression was detected by the RT-PCR technique using StepOnePlus Real-Time PCR System, the US, and SYBR Green (Takara, Japan), and gene and GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) specific primers were considered as the housekeeping genes as well. The relative genes detection for all specimens was repeated three times. The temperatures' protocol duration and the number of cycles were all set according to the SYBR Green kit (Takara, Japan). The relative expression of IL-4, IL-6, IL-10, IL-17, IFN-γ, and TGF-β genes was calculated; using the $2^{-\Delta\Delta CT}$ formula (Livak method).[20] The primers sequences which were used in the study are shown in Table 1.

Statistical Analysis

SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Kolmogorov–Smirnov tests were used to assess whether data were normally distributed. The Mann-Whitney test (for gene expression changes) and independent t-test (for comparison of demographic variables between the two groups) were used. In all tests, $p < 0.05$ was considered significant. Microsoft Excel software version 16 was used for drawing the graphs.

RESULTS

Blood samples of two patients in each group were removed from the study due to poor quality. The mean age of participants was 33.17±4.75 years. The demographic characteristics of participants are summarized in Table 2. There were no significant differences in demographic data in both studied groups.

Gene Expression Analysis

Twenty-four hours after CS, the expression of IL-10, IL-17, and IFN-γ genes was decreased in both groups. However, there was no significant difference between the two groups in terms of the rate of these changes. In addition, in the GA and SA groups (within-group), the difference in gene expression between before and 24 hours after CS was not significant (Figure 1).

Although the expressions of Il-4, IL-6, and TGF-β genes were upregulated in two groups, the differences between the groups were not significant. The mRNA levels of IL-4, 24 hours after CS, were increased in the SA group significantly (Figure 1).

Table 1. Indicates the sequences of used primers

Target gene	Forward	Reverse
IL-4	TGGGTCTCACCTCCCAACTG	**GCCGGCACATGCTAGCA**
IL-6	GCTGCAGGCACAGAACCA	**GCTGCGCAGAATGAGATGAG**
IL10	TTACCTGGAGGAGGTGATGC	**GGCCTTGCTCTTGTTTTCAC**
IL-17	AAGGCAGGAATCACAATCCC	**TGAGGTGGATCGGTTGTAGT**
IFN-γ	GGTCATTCAGATGTAGCGGA	**TCCTTGATGGTCTCCACACT**
TGF-β	CCACCTGCAAGACCATCGAC	**CTGGCGAGCCTTAGTTTGGAC**
GAPDH[*]	AACAGCCTCAAGATCATCAGC	**GGATGATGTTCTGGAGAGCC**

* Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)

Table 2. The demographic characteristics of women who underwent cesarean section in the SA and GA groups

Variable	SA (n=18) Mean ± SD	GA (n=18) Mean ± SD	P
Age (year)	32.83 ± 4.74	33.50 ± 4.87	0.680
Weight (kg)	83.72 ± 9.48	86.47± 12.99	0.473
Duration of surgery (min)	36. 11 ± 6.54	35.83 ± 8.09	0.910
Gravidity (times)	2.39 ± 0.61	2.67 ± 0.77	0.237
Gestational age (week)	39.06 ± 0.42	39.00 ± 0.48	0.715
Previous caesarean section (times)	1.17 ± 0.38	1.39 ± 0.502	0.145

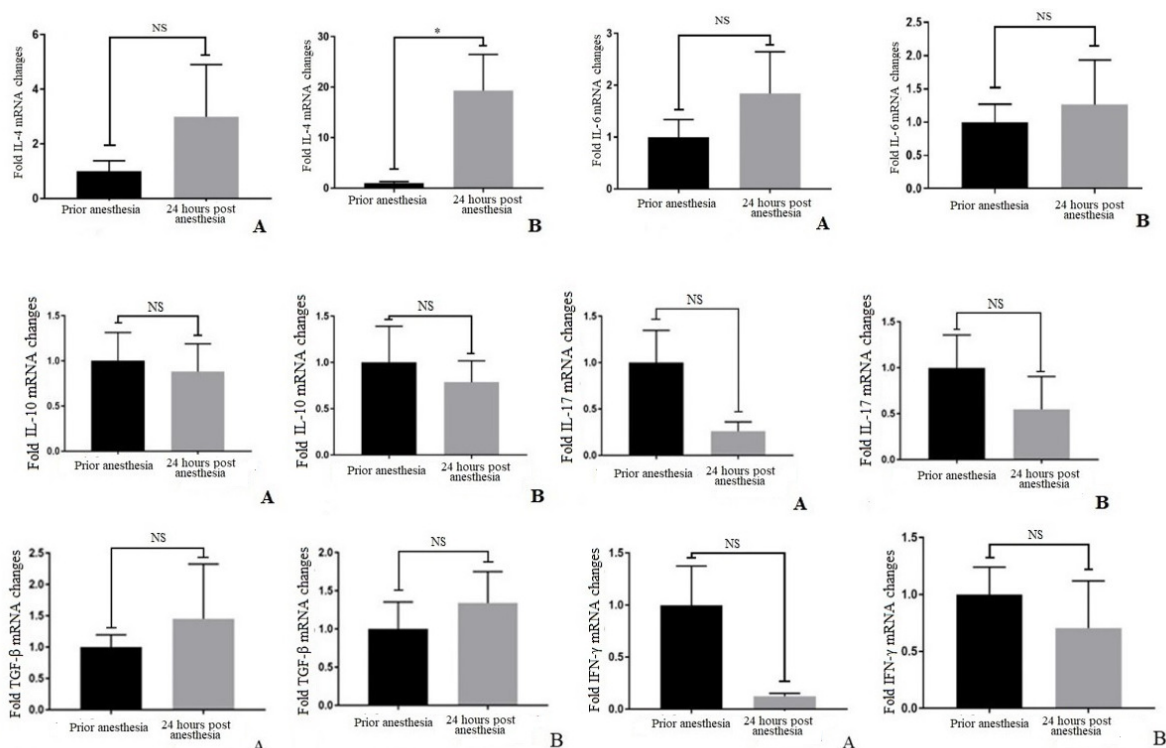SA: spinal anesthesia, GA: general anesthesia. Independent t-test

Figure 1. The mRNA expression of IL-4, IL-6, IL-10, IL-17, TGF-β, and IFN-γ in women who underwent elective Cesarean section (CS) before and 24 hours post-surgery. A: general anesthesia (n=18). B: spinal anesthesia (n=18). Data are presented as mean±SD. Mann–Whitney U test. NS= not significant. *p<0.05

DISCUSSION

All forms of anesthesia have been found to modulate the immune system and affect immunity.[21-23] In the present study, we compared the influence of general and spinal anesthesia techniques on IL-4, IL-6, IL-10, IL-17, IFN-γ, and TGF-β gene expression in peripheral blood cells. From the assay analysis, we found that these two anesthesia techniques changed the gene expression of the cytokines, but changes in the pre and 24 hours post-CS were not significant between the groups. Sufficient evidence from previous studies demonstrated that administration of anesthetics could affect expression levels of genes related to inflammatory cytokines and chemokines.[24,25]

In the present study, deregulated IL-10, IL-17, and IFN-γ genes expression were observed in patients in both groups 24 hours after induction of anesthesia and

CS. However, the expressions of Il-4, IL-6, and TGF-β genes were upregulated. Zura M and his colleagues in their study reported that IL-6 was increased in both GA and SA groups with a peak on the first postoperative day.[26] Kochiyama et al, reported that propofol (a commonly used intravenous anesthetic agent) significantly inhibited the production of IL-6 by human macrophages.[27] Although these results are different, they indicate that the immune system has changed due to anesthesia and surgery. In the present study, because there was no significant difference between the GA and SA groups in terms of the expression of cytokine gene, operative trauma is considered to have a more important role than anesthesia in altering immune system responses during surgery.[28]

The genes studied in this study, are known to participate in several processes including adaptive immunity, macrophage and neutrophil function, tissue repair, tumors growth, rejection of organ transplantations, autoimmune diseases, asthma, and allergies.[29,30] Some anesthetics can have both inhibition and potentiation effects on macrophages.[31] Immunocompetent cells release different cytokines and effector molecules, which mediate body reaction to the operation and anesthesia.[32, 33] The impaired function of neutrophils after exposure to some anesthetics was observed.[34] In a rat model of liver transplantation, sevoflurane (an inhalational anesthetic) decreased interleukin (IL)-6 levels.[35] A study comparing sevoflurane and propofol noted a similar inflammatory response, including increased IL-8 and decreased IL-17.[36]

In the present study, the mRNA level of IL-4 has increased in the SA group significantly 24 hours post-CS. IL-4 participates in tissue repair and plays pivotal roles in the regulation of the pro-inflammatory cytokines functions. Therefore, faster tissue repair and less post-CS pain (due to the anti-inflammatory effect of interleukin-4) are likely in the SA group. A limited number of studies have studied the possible effects of widely used anesthetics on wound healing.[14,37,38] Because the over-suppression of pro-inflammatory cytokines can interfere with immune system activity, identifying the effects of IL-4 gene expression upregulating requires further and more detailed studies.

The present study has several limitations. Firstly, we focused on the comparison of gene expression of IL-4, IL-6, IL-10, IL-17, IFN-γ, and TGF-β. However, studying other genes may clarify the differences between GA and SA techniques. Furthermore, it is recommended that cytokine levels be compared at different times post-anesthesia in future studies. The most important limitation of this study was the lack of follow-up and non-comparison of patients in the two groups regarding wound healing. It is suggested that a follow-up period be included in future studies to test this hypothesis.

Because the results showed the increased levels of IL-4 mRNA following CS in the SA group, it may be concluded that SA probably has a modest effect on the immune system, but more studies are warranted to obtain more distinct and definitive results.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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