The effect of Spinal and General Anesthesia on Serum Lipid Peroxides and Total Antioxidant Capacity in Diabetic Patients with Lower Limb Amputation Surgery

Short title: The effect of Anesthesia on Serum Lipid Peroxides and Total Antioxidant Capacity

Author contributions: All authors contributed extensively to the work presented in this paper.

Key words: antioxidant, lipid peroxide, general anesthesia, spinal anesthesia, diabetic foot, foot amputation

This article includes 3044 words, 2 tables, 1 figure and 18 references.

Abstract

Background: Anesthesia is performed in two major methods including regional and general.

Aim: The aim of this study was to compare the effect of anesthesia method (spinal and general) on oxidative stress in diabetic patients underwent diabetic amputation surgery.

Design and Setting: randomized control trial

Method: In this randomized control trial, 40 patients with diabetic foot who were candidate for foot amputation surgery at our academic hospital, in 2013 were selected and divided into two groups based on anesthesia method. Lipid peroxide level and serum total antioxidant capacity (TAC) were measured before anesthesia induction and one hour after surgery. As the normal range, the findings obtained from 23 healthy volunteers were utilized.

Results: mean age was 54.9±11.21 and 52.4±11.23 years in the spinal anesthesia (SA) and the general anesthesia (GA) group, respectively (p=0.49). Serum TAC in GA group increased from 1.03±0.04 mM to 2.98±0.7 mM, in SA group the increase of serum TAC from 1.22±0.11 mM to 3.42±0.5 mM was observed; that indicated the increases of serum TAC in

both groups was not significantly different (p =0.21). Serum Malondialdehyde (MDA) in GA and SA groups did not show a significant difference before surgery (31.14±3.9 mM vs 29.06±2.49 mM in GA and SA groups, respectively) (p =0.31), it was significantly different after surgery (23.14±2.6 mM and 19.24±2.7 mM in GA and SA groups, respectively) (p =0.03).

Conclusion: lower limb amputation can help to control oxidative stress in diabetic patients; and considering serum MDA as a marker of oxidative stress, SA seems to be more effective to control this problem.

Registration code: Iranian Registry of Clinical Trials code: IRCT201402208384N3

Key words: antioxidant, lipid peroxide, general anesthesia, spinal anesthesia, diabetic foot, foot amputation

Introduction

There are a variety of studies which investigated the differences between general anesthesia (GA) and spinal anesthesia (SA) concerning different aspects such as patient satisfaction (1), cost-effectiveness (2,3), postoperative hospital stay (4), postoperative pain scores and analgesic requirements (5,6), acid-base status (7), as well as their effects on serum concentration of pro-inflammatory and anti-inflammatory cytokines (8). In 2011, for example, Mas *et al* compared plasma isofurans and F (2)-isoprostanes, as reliable markers of oxidative stress, in spinal versus general anesthesia in patients undergoing knee-replacement surgery, and they reported increased oxidative stress during GA (9).

Oxidative stress is a redox perturbation which results from the imbalance between oxidants and antioxidants in favor of the former (5). Human body is always exposed to the oxidizing effects of free radicals and oxidants which originate both endogenously and exogenously. On the other hand, since a reduced intracellular environment is essential for cell survival, human body is equipped with a number of antioxidants which maintain and keep the redox homeostasis (3). Based on the fact that oxidative stress can oxidize and damage cellular proteins, lipids and nucleic acids, there are a lot of evidences which approve the role of oxidative stress in pathogenesis of a number of diseases such as cancers, autoimmune diseases, neurodegenerative disorders (8, 9), cardiovascular diseases such as myocardial infarction and atherosclerosis(6).

Moreover, there are a number of studies showing that anesthesia can lead to oxidative stress (4, 8). This effect can be due to the drug effect. Desflurane is an example which weakens the antioxidant levels in the blood of operative patients (2). On the other hand, the depth of anesthesia is reported to be another factor that can influence oxidative stress (3).

Besides the fact that most of the above-mentioned studies were oriented towards the clinical rather than molecular differences between two types of anesthesia, there is not any study to investigate the difference between general and spinal anesthesia on the subsequent oxidative stress. Therefore, in this study, we aimed to evaluate how general and spinal anesthesia affect serum lipid peroxides and total antioxidant capacity (TAC), as two main indicators of redox homeostasis, and how they differ regarding these effects in lower limb amputation in diabetic patients. The aim of this study was to evaluate if the anesthesia method (spinal and general anesthesia) can affect lipid peroxidation and total antioxidant capacity in diabetic patients underwent diabetic amputation surgery.

Material and Methods

Study design and participants

This randomized clinical trial (Iranian Registry of Clinical Trials code: IRCT201402208384N3) study was performed on 40 diabetic patients with diabetic foot who were candidate for lower limb amputation and admitted to our academic hospital in 2013. The study was ethically approved by the ethical Committee of Mashhad University of Medical Sciences (code of approval: 910198), Mashhad, Iran; and an informed consent was obtained from our patients before involving them in the research. The patients who did not like to join this study as well as those who were suffering from a simultaneous severe disease such as cancers and recent cardiovascular disease were excluded from this project. Besides, in order to use a similar method of surgery for all of our patients which were posterior flap in this study, ischemia was the only indication of amputation that was included in this investigation. Patients with lower limb amputation due to infection or osteomyelitis for which another surgery method had been used were excluded from this study. We used random block method for randomization.

We conducted this study on 20 diabetic patients, who were candidates for amputation. The participants were randomly assigned into two groups regard to random block method with computerized generated randomization sequence of 20 in either GA or SA groups (figure-1). This study was conducted as a single blind study and the biochemist was blind about the anesthesia method.

Figure-1: consort flow diagram of patients follow up

Procedures and variables assessment

From each patient, two blood samples of 5 ml were taken from brachial vein immediately before anesthesia and 1 hour after the surgery. In each case, serum was separated and stored at -80 °C until laboratory analysis. For patients in GA group, a similar protocol was utilized in which 0.04 mg/kg Midazolam and 3µg/kg Fentanyl were prescribed before anesthesia; thereafter, anesthesia was induced using 2 mg/kg Propofol and 0.5 mg/kg Atracurium, and maintained using 100 µg/kg/min and 50 µg/hour Fentanyl plus 10 mg/30 min Atracurium.

For patients in SA group, 3 ml Bupivacaine 0.5% was injected into the subarachnoid space of the spinal canal between the third and fourth lumbar space or between the fifth lumbar and first sacral space of the spinal column. A maximum level of the sensory block, defined as decreased sensation, at the level of T8 was confirmed before surgery.

For lipid peroxides, the concentration of malondialdehyde (MDA) was measured that is the main product of lipid peroxidation. We evaluate amputation in diabetic patients because in our hospital traumatic and other causes of amputation were rare.

The serum Total Antioxidant Capacity (TAC) of samples was measured using Antioxidant Assay Kit (Item Number 709001) purchased from Cayman Chemical, USA. The assay was performed according to the instructions provided by the company; which is in brief based on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) to $ABTS^{®\cdot+}$ by metmyoglobin. The capacity of serum antioxidants is compared with that of Trolox, a water-soluble tocopherol analogue, and is presented as molar Trolox equivalents (10).

The serum level of Malondialdehyde (MDA) as the main lipid peroxide was measured using TBARS (Thiobarbituric Acid Reactive Substances) Assay Kit (Item Number 10009055) purchased from Cayman Chemical, USA. The assay was performed according to the instructions provided by the company. In brief, the assay is based on the formation of MDA-TBA complex under high temperature (90-100°C) and acidic conditions that is colorimetrically detectable at 530-540 nm (11).

Statistical analysis

Appropriate statistical methods were applied in SPSS 11.5, and data was presented as Mean ± SD. Variables were evaluated for normality by kolmogorov smirnov test and Chi square test was performed for qualitative variables, paired t-test and Student t-test was used to compare variables with normal distribution, Mann Whitney test used for other quantitative variables. A *P*-value of ≤ 0.05 was considered significant.

Demographic characteristics of patients summarized in table-1.No significant difference was observed between groups regard to drug used for diabetes mellitus (*P*=0.587).

Table-1: Demographic characteristics of patients

	GA	SA	P value
Male (n/%)	4 (40)[a]	6 (60) [a]	0.600[*]
Female (n/%)	6 (60) [a]	4 (40) [a]	
Age (mean± SD)	52.4±11.23 [b]	54.9±11.21 [b]	0.49[**]
Diabetes duration (yrs)	10.6±4.4 [b]	11.2±5.9 [b]	0.841[**]
Surgery duration (min)	64.1±6.7 [b]	101.5±8.9 [b]	0.003[**]

a: number/percentage

b: mean± SD

*: chi- square

**: Mann Whitney

Results

As it is shown in Table 2, there was not any significant difference between GA and SA groups concerning the drug which they were using for diabetes mellitus (*P*-value=0.59). Moreover, the average duration between diagnosis of diabetes mellitus and amputation in two groups was not significantly different (10.6±4.5 and 11.2±5.9 years in GA and SA groups, respectively) (*P*-value=0.84). There were no difference between man and woman level of these antioxidant lipid peroxide before (*P*<0.05) and after (*P*<0.05) anesthesia.

In all patients in both groups, standard posterior flap technique was utilized. Based on statistical analysis, there was not a significant difference between two groups with respect to the duration of surgery (84.5±8.9 min and 81.4±6.7 min in GA and SA groups, respectively) (*P*-value=0.73).

Mean TAC level in control group was 1.17±1.01 mmol/lit and mean MDA was 3.12±0.95 M/mlμ.Oxidative stress indexes compared before and after surgery in GA and SA patients in table-2.

Table-2: MDA and TCA levels in both groups before and after surgery

		Before surgery	After surgery	*P* value *
GA	MDA (M/mlμ)	31.14±3.9	23.14±2.6	<0.001
	TAC (mmol/lit)	3.18±1.7	0.78±0.043	<0.001
SA	MDA (M/mlμ)	29.06±2.49	19.24±2.7	<0.001
	TAC (mmol/lit)	3.42±1.3	1.23±0.11	<0.001

*: T.test

TAC levels comparison showed no significant difference between GA and SA groups before surgery (*P*=0.850). This index reduced after surgery, and TAC was lower in GA patients than SA ones (*P*<0.001). Mean MDA level in GA and SA groups was not differ before surgery (*P*=0.610), but this difference was significant after amputation (*P*=0.031).

Although TAC level after amputation in GA group was lower than control (*P*<0.001), there was no significant difference between SA (after surgery) and controls (*P*=0.653). MDA levels after surgery were higher in both GA and SA groups in comparison with controls (P<0.001).

Discussion

The aim of this study was to investigate the effect of two anesthesia method (GA and SA) on oxidative stress in diabetic patients. This was the first study on human, to best of our knowledge. In order to achieve this goal and since it is reported that anesthesia can lead to redox perturbation and subsequent oxidative stress (9, 10, 11,12), serum TAC and lipid peroxides were measured as two well-known markers of oxidative stress.

Our project revealed that oxidative stress indexes (TAC and MDA) change after limb amputation in diabetic patients. TAC reduced significantly in patients underwent limb amputation with GA, but MDA level reached near normal levels after surgery. This means, although diabetic patients are at more risk for oxidative stress, using GA could exacerbate it.

Lee and colleagues showed that surgery in traumatic injuries increases the oxidative stress (13). This difference might be occurred due to the study project as an animal model, and measuring different oxidative stress indexes. In our study we evaluated patients after limb amputation, which might lead to washout oxidative stress indexes more rapidly in comparison with other surgeries. Since ischemic or infected diabetic foot is a source of free radical production (14), its removal via surgery may help to preserve the antioxidant capacity of serum.

Bravo-Cuellar showed that the type of anesthesia influences inflammatory indexes and oxidative stress, and cytokines and oxidative stress in GA were higher than regional anesthesia group, 24 hours after surgery (15). Bedirli demonstrated that epidural anesthesia attenuated cytokine and malondialdehyde levels and increased the antioxidant enzyme (16). This finding confirmed our results, serum TAC in our patients before surgery which was significantly lower compare to the healthy control group, reached to the normal range after surgery that empowered this hypothesis. At the same time, lipid peroxides showed a decline in both groups after surgery; which was in accordance to this hypothesis the removal of diabetic foot helps to overcome the oxidative stress from which the patient is suffering. The magnitude of this change in SA group, however, was greater compare to GA group; this can be considered as an advantage for using SA rather than GA when it is applicable.

Despite the decrease of serum MDA, as a representative of lipid peroxides, after surgery, it was still much higher in our patients compare to our healthy control group; which could be due to a great redox perturbation that results from diabetes mellitus (17, 18). Moreover, there are evidence that anesthesia effect on oxidative stress can be lasted for 24 hours or more (16), but we measured its indexes right after surgery.

The main limitation of our study was the difficulty to assign TCA and MDA changes to drugs and matching administered drugs before surgery. And we could not match both group regard to diabetes control and severity. We did not consider the type of diabetes of patients enrolled in their study, because of our small sample size and as a pilot study the patients were not matched for variables like duration of anesthesia, bleeding, comorbidities.

Conclusion

Therefore, based on this study, we concluded that lower limb amputation, when it is indicated, can help to control redox perturbation and oxidative stress in diabetic patients; and considering serum MDA as a marker of oxidative stress, SA seems to be more effective to control this problem.

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Authors' contribution: This work was carried out in collaboration between all authors.

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