THE EVALUATION OF ISCHEMIA/REPERFUSION INJURY ON REGIONAL AND INHALATION ANAESTHESIA DURING ARTHROSCOPIC KNEE SURGERY UNDER A TOURNIQUET

ARTROSKOPİK DİZ CERRAHİSİNDE TURNİKE UYGULAMASINA BAĞLI İSKEMİ REPERFÜZYON HASARINA İNHALASYON VE REJYONEL ANESTEZİ UYGULAMARININ ETKİLERİ

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ABSTRACT

OBJECTIVE: *The application of a tourniquet for knee surgery is often used to improve exposure operative field. However, the release of tourniquet causes an ischemia/reperfusion injury. The aim of the study was to investigate the effect of spinal blockade and sciatic-femoral somatic blockade, sevoflurane and desflurane anaesthesia, on oxidative stress by determining malondialdehyde (MDA) and nitric oxide (NO) levels in blood and synovial tissue during arthroscopic knee surgery under a tourniquet.*

MATERIAL AND METHODS: *We studied 60 adult ASA I-II patients undergoing arthroscopic knee surgery under a tourniquet were divided into four groups. It was performed sciatic-femoral blockade in Group I and spinal anaesthesia in Group II. General anaesthesia was used with sevoflurane and desflurane, in Group III and Group IV respectively. Venous blood samples were obtained before anaesthesia (T1), 1 min before tourniquet release (T2), 5 min after tourniquet release (T3) and 20 min after tourniquet release (T4). In addition synovial membrane tissue samples were obtained the periods of T2 and T3.*

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RESULTS: *Plasma concentrations of MDA increased significantly in all groups at T3 value compared with the T1 value. In addition 20 min after reperfusion (T4) plasma MDA levels were significantly increase than T1 period in Group I and IV. The tissue MDA levels were higher than ischemia in all groups the early reperfusion periods. The NO levels were significantly increased at the T2, T3 and T4 periods compared to T1 in all groups. There was a significant increase in tissue NO in Group I.*

CONCLUSION: *According to our results showed that temporary neutrophil activation and transendothelial neutrophil migration during during arthroscopic knee surgery under a tourniquet. Spinal block and sevoflurane anaesthesia may be preferred to desflurane anesthesia and sciatic-femoral block techniques usage in cases where ischemia-reperfusion is created in the extremities.*

Keywords: *Arthroscopy, Ischemia, Reperfusion Injury, Anesthesia, Inhalation, Anesthesia, Spinal.*

ÖZ

AMAÇ: *Artroskopik diz cerrahisinde cerrahi alan görüşünü arttırmak amacıyla sıklıkla turnike uygulanmaktadır. Bununla beraber turnikenin indirilmesi ile beraber iskemi/reperfüzyon hasarı gelişmektedir. Bu çalışmanın amacı turnike altında gerçekleşen artroskopik diz cerrahinde spinal blok, siyatik-femoral blok, sevofluran ve desfluran anestezi uygulamasının kan ve snovial dokudaki MDA ve NO sevilerini değerlendirmektir.*

GEREÇ VE YÖNTEMLER: *Çalışmaya ASA I-II 60 erişkin hasta dahil edilerek turnike altında artroskopik cerrahi geiçrecek hastalar drt gruba ayrıldı. Hastalara siyatik-femoral blokaj (Grup I) ve spinal blok (Grup II) uygulandı. Genel anestezi uygulaması sırasıyla sevofluran (Grup III) ve desfluran (Grup IV) ile gerçekleştirildi. Anestezi öncesi (T1), turnike açılmadan 1 dk. önce (T2), turnike açıldıktan 5 dk. sonra (T3) ve turnike açıldıktan 20 dk. sonra (T4) kan örnekleri alındı. Ek olarak T2. ve T3. evrelerde cerrahi alandan snovial doku örnekleri alındı.*

BULGULAR: *Çalışmamızda tüm gruplardaki MDA düzeyleri T3 evresinde, T1 evresine göre anlamlı artış göstermiştir. Ek olarak, Grup I ve IV'de T4 evresindeki plazma MDA düzeyleri T1 evresine göre anlamlı artış göstermiştir. Tüm gruplardaki plazma NO düzeyleri T2, T3 ve T4 evrelerinde; T1 evresine göre anlamlı artış göstermiştir. Doku NO düzeyleri, Grup I'de en belirgin olmak üzere artmıştır.*

SONUÇ: *Çalışmamızda artroskopik cerrahide turnike uygulaması sonrasında geçici nötrofil aktivasyonu ve transendotelial nötrofil migrasyonu gösterilmiştir. Turnike kullanımına bağlı iskemi reperfüzyon hasarında spinal blok ve sevofluran ile anestezisi uygulamasının desfluran ve siyatik-femoral blok tekniğine göre tercih edilmesi gerektiği sonucuna varıldı.*

Anahtar Kelimeler: *Artroskopi; iskemi; Reperfüzyon hasarı; Anestezi, inhalasyon; Anestezi, spinal*

INTRODUCTION

Ischemia is the condition suffered by tissues when deprived of blood flow mostly the effects of inadequate oxygen. Reperfusion injury refers to the tissue damage inflicted when blood flow is restored after an ischemic period. It has been shown that Reactive Oxygen Species (ROS) are generated during ischemia-reperfusion (I/R) injury and known to originate from intracellular sources of the ensuing pathological complications (1,2). Arterial tourniquets are used for extremity surgery to reduce blood loss and provide good operating conditions. For this reason, the use of a tourniquet for knee surgery may result in I/R injury when perfusion is re-established. This procedure causes hemodynamic and metabolic changes whose entity depends on the tourniquet inflation-deflation phase, the time on tourniquet inflation, the extension of area and the anaesthesia method (general, spinal or epidural) (1,3-5). Massive oxygen free radicals after I/R injury following tourniquet release followed by endothelial dysfunction or neutrophil infiltration, free radicals induce peroxidation of cell membrane macromolecules, triggers the oxidative damage (6,7). Lipid per oxidation is a chain reaction leading to oxidation of polyunsaturated fatty acids, disrupting membrane structure and producing toxic metabolites. Base damage products carbonyls, and other amino acid modifications such as malondialdehyde (MDA) were used evaluate the oxidative damage on DNA, protein, and lipid (8).

Nitric oxide (NO), another free radical, is a highly reactive free radical with a multitude of organ specific regulatory functions. NO has been speculated to be both ctotoxic and cytoprotective in ischemic injury (9). Wong CH et al. demonstrated that sevoflurane significantly increases intracellular H2O2 and/or peroxide, superoxide and NO in polymorph nuclear neutrophils within 1 hour treatment (10). Superoxide rapidly reacts with NO and forms peroxinitride. Peroxinitride is also one of the potent reactive metabolites for the initiation of lipid peroxidation (11).

The effects of volatile anaesthetics and spinal anaesthesia on lipid per oxidation have been studied, but no study comparing sevoflurane, desflurane, spinal anaesthesia and sciatic-femoral somatic blockade in human beings. The purpose of the clinical study was to investigate the effects of sevoflurane, desflurane anesthesia, spinal and siyatic-femoral blockade on I/R injury with plasma and synovial tissue MDA and NO levels evaluation.

MATERIAL AND METHODS

After obtaining Ethics Committee approval and informed consent, 60 adult, ASA I-II patients were investigated undergoing arthroscopy using a tourniquet. Patients were allocated randomly by closed envelope method to one of four groups: siyatic-femoral blockage (Group I), spinal blockade (Group II), sevoflurane anesthesia (Group III) and desflurane anesthesia (Group IV). Patients with metabolic, renal or hepatic disturbances and those taking antioxidant agents were excluded from the study.

On the day study, all patients were premedicated with atropine intramusculary before 45 minutes the induction of anesthesia. Cardio respiratory monitoring including heart rate (HR), finger tip puls oximetry for SpO2, non invasive blood pressure (Systolic, diastolic and mean arterial pressure; SAP, DAP, MAP), end tidal carbon dioxide (ETCO2), desflurane and sevoflurane concentrations were continuously intraoperative monitored. A 20 G i.v. cannula was inserted and connected to an infusion of saline.

In Group I, received single femoral (3-in-1) and sciatic blocks with insulated 22 gauge regional needles

(Stimuplex®, Braun) attached to a peripheral nerve stimulator. The femoral nerve was identified by eliciting quadriceps contractions ('dancing patella') at a current setting below 0.5 mA. The sciatic block was undertaken using the classical Labatt approach (12). The sciatic nerve was identified by eliciting foot movements (dorsiflexion or plantar flexion) below 0.5 mA. Thirty milliliters of bupivacaine 0.375% was used for the femoral component and 25 ml of bupivacaine 0.375% for the sciatic component. In Group II, intrathecal anesthesia by 0.5 % bupivacaine 10-12 mg was performed. The patients of Group III and IV received 1 μgkg-1 fentanil and 5-7 mgkg-1 thiopental for induction of anesthesia. Tracheal intubation was facilitated by vecuronium 0.1 mgkg-1 intravenously. Ventilation was maintained with 50 % N2O in oxygen, 10 mlkg-1 tidal volume and 10 breats min-1 and end-tidal CO2 was kept within 34-37 mmHg. Volatile anesthetics concentrations were adjusted to maintain sevoflurane 1 MAC in Group III and desflurane 1 MAC in Group IV with systolic blood pressure within ± 20 % of baseline. A tourniquet was applied at a pressure approximately twice the systolic arterial blood pressure. Fluid deficits were corrected with 0.9 % NaCL during surgery and no blood was given. After surgery, neuromuscular blockade was reversed by 0.01 mg-1kg-1 atropine and 0.05 mg-1kg-1 neostigmine, and then the patients were extubated. Mean arterial pressure was maintained at 70-90 mmHg and peripheral oxygen saturation was above 96 % throughout the operation.

Blood samples for ischemia-reperfusion injury were also obtained before anaesthesia (T1), 1 min before tourniquet release (T2), 5 min after tourniquet release (T3) and 20 min after tourniquet release (T4). In addition synovial membrane tissue samples were obtained the periods of T2 and T3.

The blood samples were immediately centrifuged (1500 rpm) within 10 min and supernatants were stored at -70 oC. The synovial membrane tissue samples were stored at -80 oC until analysis. After washing with 0.9% NaCL, tissue samples were homogenized in 0.02 M EDTA using mechanic homogenization (Art-Micra D-8, Manheim, Germany). Samples were kept in ice bath during the study and centrifuged (5000 g for 10 min at 4°C) after homogenization. The supernatant was used for all analysis. Protein level was measured using Lowry's method (13). TBARS levels indicated of lipid per oxidation were measured as described by Armstrong and al-Awadi, who modified the Yagi method (14). The calibration curve, was prepared with 1, 1, 3, 3-tetramethoxypropane (Sigma, USA). The results were calculated as nmolg-1 protein. Nitrite/nitrate levels were measured as described by Miranda et al.15 Nitrate was reduced to nitrite with vanadium and then nitrite level measured by using Griess reagents. This reflects the total amount of nitrate and nitrite in the sample. Serial dilutions of Na nitrate (Merck, Germany) were used as standards and the results were expressed as μmolg-1 protein.

Data were given as mean ± standard deviation. Patients characteristics, surgery time, tourniquet time were performed using the Kruskal-Wallis tests. The blood and tissue oxidative stress status were detected using Kruscall Wallis test to compare four groups, a P- value of less than 0.05 was considered statistically significant. Mann Whitney-U test was used in two group comparisons. Friedman variance analysis was used for comparison within the groups ($p<0.05$), followed by the Wilcoxan test was applied for double periods and $p < 0.005$ was used to establish statistical significance.

RESULTS

There were no statistically significant differences among the groups in age, weight, duration of surgery and tourniquet application (Table 1). Patient's baseline values of blood MDA and NO were not statistically different between the groups (Table 1).

Table 1: 1. Patients characteristics (Mean ± SD)

Group	Group I	Group II	Group III	Group IV
N	15	15	15	15
Age (year)	46 ±12.5	42.9±13.4	39±10	43±14.3
Weight (kg)	78.2±6.8	84.1±12.7	82.2±12	77.2±13
Sex (M/F)	7/8	9/6	8/7	7/8
Surgery time (min)	61.5±10.2	57.26±7.9	55.4±10.7	58.1±12.6
Tourniquet time (min)	53.3±7.6	52.2±7.3	49.8±9.9	54.4±9.4

Plasma concentrations of MDA increased significantly in all groups at T3 value compared with the T1 value. But 20 min after reperfusion (T4) plasma MDA levels were significantly increase than T1 period in Group I and Group IV only (Table 2).

Tissue MDA levels were also increased significantly in the T3 in all groups compared to T2 period (Table 2).

Table 2: Blood MDA and NO levels in both groups (µmolL-1) (Mean ± SD)

Periods		Groups	Group I	Group II	Group III	Group IV
T1	MDA		4.83	3.41	3.73	4.52
T2	MDA		6.44	4.82	3.3	5.83
T3	MDA		7.18[a]	5.47[a]	4.27[a]	6.11[a]
		P	*0.004*	*0.031*	*0.014*	*0.013*
T4	MDA		6.01[b]	3.54	4.31	4.78 [b]
		P	*0.031*			*0.026*
T1	NO		21.34[c]	23.24[c]	29.76[c]	19.66[c]
		P	*0.034*	*0.036*	*0.016*	*0.003*
T2	NO		34.37	28.58	50.24[d]	44.42[d]
		P			*0.007*	*0.001*
T3	NO		34.34	32.27	52.63[d]	42.49
		P			*0.001*	
T4	NO		33.26	37.16	47.73	46.8

a T3 values vs. T1 values
b T4 values vs. T1 values
c T1 values vs. T2,T3,T4 values
d T2 values vs. Group II values

The NO levels were significantly higher at T2, T3 and T4 compared to T1 in all groups. The NO levels were significantly higher at T2 and T3 in Group III and only at T2 in Group IV compared to Group II (Table 2). There was a significant increase in tissue NO levels at the early reperfusion period (T3) compared to the ischemia (T2) period, most marked in Group I.

Table 3: Tissue MDA (nmolg-1) and NO levels (µmolg-1) in both groups (Mean ± SD)

Periods	T2	T3		T2	T3	
	Tissue MDA	Tissue MDA		Tissue NO	Tissue NO	
Groups			P			P
Group I	6.08	8.29[a]	0.03	68.67	382.78[a,b]	0.006 0.001
Group II	4.03	6.35[a]	0.012	53.01	151.28[a]	0.01
Group III	5.6	6.45[a]	0.011	62.43	155.69[a]	0.017
Group IV	4.5	6.95[a]	0.014	59.11	146.8[a]	0.02

a T3 values vs. T2 values
b T3 values vs. other groups

DISCUSSION

Many clinical conditions, such as atherosclerosis, carcinogenesis and I/R injury are releated ROS production. I/R injury commmanly encountered under clinical conditions. This procedure is associated with the use of pneumatic tourniquets during arthroscopic knee surgery. Ischemia in the extremities is associated with lipid peroxydation, an autocatalytic mechanism leading to ROS and cell death (2, 15, 16). The main targets of ROS are the polyunsaturated fatty acids in cell membranes causing lipid peroxydation and MDA formation, which lead to damage to cell structures and function. Damage from oxygen free radicals after reperfusion has been documented in many different tissues, including skeletal muscle. MDA is good indicator of the degree of lipid per oxidation and more stable, longer living degradative product of lipid peroxides (17).

The role of anesthetics in I/R is of interest. The studies of to compare the effects of sevoflurane and desflurane anesthesia on lipid per oxidation, desflurane

appears to cause more systemic and regional lipid peroxydation than sevoflurane during laparoscopic cholesystectomy in healthy human beings (18). Sıvacı et al. assessed the effects of low flow sevoflurane and desflurane anaesthesia during laparoscopic surgery (17). Their data indicate that desflurane has appeared to induce oxidative stress whereas sevoflurane did not (there was a significant increase in the 6th and 24th hour plasma MDA levels in the desflurane group compared the sevoflurane group). Saricioglu et al. were to investigate the effect of low dose n-acetyl cysteine (NAC) infusion on oxidative stress by determining blood and synovial tissue MDA levels during arthroscopic knee surgery, with desflurane anesthesia. This study results showed that blood MDA levels were significantly higher in the control group at the preischemia and reperfusion periods and in both groups tissue concentrations decreased significantly during ischemia, increased significantly during reperfusion (19). An other study evaluated the circulating and lung oxidative status during general anesthesia established with propofol, sevoflurane and desflurane in mechanical ventilated swines (20). Their results show that animals exposed to 1 MAC desflurane have increased MDA concentrations both in serum and bronchoalveoler lavage. They stress the increase of proinflammatory cytokines as a factor in the oxidative stress-increasing effect of desflurane. In the present study, we observed similar changes in MDA levels in all groups at ischemia period but the MDA increase in the desflurane group persisted in the late reperfusion period. Thus desflurane caused more lipids per oxidation than sevoflurane.

The reason for choosing spinal anesthesia was the fewer that less hemodynamic and metabolic changes were seen with the tourniquet in patients undergoing spinal anesthesia compared with general anesthesia. Spinal anesthesia reduce the stress-including hormones such as adrenaline, noradrenalin and cortisol (21, 22). Oxidative damage is reported to be less in babies born with C/S under spinal anesthesia than those born with general anesthesia (23). However, Ya Jung Cheng et al. have reported a marked increase in the ROS products 1 minute before and 5 and 20 minutes after reperfusion compared to basal value in patients undergoing spinal anesthesia with a below-knee tourniquet (24). Spinal anesthesia have done an increase in serum MDA at the ischemia and reperfusion stages (5 minutes) in patients undergoing arthroscopy (19). However, the increase in the reperfusion period was approximately 8 times the basal value. Turan et al. have reported that the decreased MDA levels in the spinal group may be due to the added propofol infusion (25). In our study, there was no increase in blood MDA levels in the spinal anesthesia group at the late reperfusion although the MDA levels increased in both serum and tissues at the early reperfusion stage. We believe this is an important result. Studies have reported that the ROS increase is maximum 5 min after reperfusion and starts to decrease at 20 minutes (24). Spinal anesthesia administration has therefore been found advantageous due to the lack of an increase in MDA and NO levels at the late reperfusion period.

NO plays a major role in many organ systems. There are conditions in which it will be beneficial to increase NO and other conditions in which selective inhibition of NO formation may be desirable. Ozokutan et al. were used to increase NO synthesis in the I/R injury process in testes (26). Their data suggest that NO acts as a free radical and inhibition of NO production could prevent reperfusion injury. Bagdatoglu et al were carried out to evaluate the role of lipidperoxidation, NO and fibronectin in an experimental model of peripheral nerves. These study showed that serum levels of MDA increased in the ischemia period. But serum levels of MDA decreased in the reperfusion periods when NO simultaneously increased (27). We observed increased NO at the

ischemia and early and late reperfusion period in all groups in our study. The increase in tissue NO levels was most marked in the siyatic-femoral blockage group in the early reperfusion period.

Reperfusion of the acutely ischemic limb may, paradoxically, lead to systemic complications that account for significant morbidity and mortality. According to our results, spinal anesthesia and sevoflurane anesthesia may be preferred to desflurane usage and sciatic-femoral block techniques in cases where I/R is created in the extremities.

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