EFFECTS OF ANESTHETIC DRUGS (KETAMINE, PROPOFOL OR COMBINATION OF BOTH) ON TOURNIQUET-INDUCED ISCHEMIA-REPERFUSION INJURY DURING KNEE SURGERY

Doaa Mohamed Almonayery Ibrahim[1*], Hanaa A M Khalil[1], Ahmed Sayed Ahmed Hussein Elshamy[2], Mohamed Ramadan Elsayed Elfeshawy[2]

ArticleHistory:Received:21.04.2023 **Revised:**26.05.2023 **Accepted:**01.06.2023

Abstract

Background: To ensure a safe and bloodless operating environment during lower limb surgery, a proximal tourniquet is frequently employed. After the tourniquet is loosened, oxygen is rushed back into the ischemic tissue, releasing a flood of free oxygen radicals into the body's bloodstream. peroxidizing macromolecules in cell membranes, including polyunsaturated fatty acids and plasma lipoproteins.

Aim: research on the effects of using ketamin, propofol, or a combination of both on tourniquet-induced ischemia reperfusion damage during knee surgery under spinal anathesia.

Patients and Methods: Sixty patients of both sexes and ages 20-60years old took part in the study, which took place at AL Zahraa Hospital on the campus of Al Azhar University. the ketamine group (groupI) receives an infusion of 0.5 mg/kg/h of ketamine. in propofol group (group II) an intravenous dose of two mg/kg/h of propofol is given to patients Group III patients recived an infusion of 2 mg/kg of propofol in addition to 0.5 mg/kg of ketamine per hour.

Results: There were no significant variations amongst groups in age, sex, mass, height, period of operation, tourniquet time, or ASA physical status, HR, ABGI, or ABGII. When comparing the baseline value to the remaining times, in all groups and RSS showed statistically significant differences.

Conclusion: During the ischemic phase, MDA levels were dramatically decreased by both ketamine and propofol, which were given to reduce the risk of ischemia-reperfusion damage in skeletal muscles due to tourniquet application. But when used combined, they exhibit the best outcomes, indicating that the ketamine-propofol combination is helpful for preventing ischemia-reperfusion injury. During the ischemia period, catalase activity was highest at the 5th and 30th minute after reperfusion. Group III has more going on than Group II or Group I.

Keywords:ketamine, propofol, RSS, and tourniquet-induced ischemia-reperfusion

[1]Department of anesthesia and intensive care, faculty of medicine for girls, Al-Azhar University
[2]Department of orthopedic surgery, faculty of medicine for girls, Al-Azhar University

DOI: 10.31838/ecb/2023.12.6.76

1. INTRODUCTION

To ensure a safe and bloodless operating environment during lower limb surgery, a proximal tourniquet is frequently employed. Ischemia reperfusion damage, however, can occur if the artery supplying the knee is temporarily blocked during arthroscopic surgery, and then re-perfused once the tourniquet is released. After the tourniquet is loosened, oxygen is rushed back into the ischemic tissue, releasing a flood of free oxygen radicals into the body's bloodstream. Macromolecular peroxidation of polyunsaturated fatty acids and plasma lipoproteins. When cells are subjected to oxidative stress, lipids, proteins, and DNA are all damaged. Furthermore, harmful metabolites such malondialdehyde (MDA) are produced.[1]

Ischemia-reperfusion injury (IRI) may be caused by a number of different things, including the generation of free oxygen radicals & subsequent lipid peroxidation, cell death by apoptosis or necrosisdamage to the microvasculature, and inflammatory cytokines Malondialdehyde (MDA) is a result of the lipid peroxidation of cell membranes induced by reactive oxygen species(ROS) & is a sensitive marker of ischemia-reperfusion damage. that arise with reperfusion injury. ROS levels and the ability of antioxidant enzymes to scavenge them are in equilibrium. Since antioxidants are used up during oxidative stress, the oxidation bulk and the intake level of antioxidants together contribute to the total antioxidant capacity (TAC). Before ischemia, during ischemia, and at the beginning of

reperfusion are the three windows of opportunity to prevent ischemia-reperfusion harm.[2]

Because of its analgesic and anti-inflammatory characteristics, ketamine has been advocated for usage in circumstances characterised by inflammation. The organs are shielded from damage caused by ischemia and reperfusion because to their anti-inflammatory capabilities.[3]

Antioxidants with free radical scavenging abilities can limit tissue damage brought on by lipid peroxidation. Antioxidants like propofol are one of them.

Propofol lowers the damage caused by free radicals, has a pro-inflammatory cytokine response, and has a stronger antioxidant effect.[4]

Studies in both the laboratory and the operating room have shown that anaesthetic drugs and techniques can mitigate IRI, hence reducing the risk of tissue harm. Multiple studies in animal models have demonstrated the preventive effect of intravenous anaesthetics against ischemia and reperfusion damage.[5]

- **Aim of Work**

This thesis set out to examine the impact of ketamin, propofol, or a combination of the both on tourniquet-induced ischemia reperfusion damage under spinal anaesthesia for knee surgery.

2. PATIENTS AND METHODS

This interest, randomised controlled research was made at Al Azhar University, AL Zahraa Hospital on 60 patients of both sexes, ages 20–60, ASAI and ASAII physical status, who were undergoing elective arthroscopic knee surgery for meniscal & chondral lesions. The research was accepted by local ethical committee and Informed permission was gained in all cases. The patients were split into three groups of twenty people (N=20) with similar demographics based on the medication they received. Following the collection of baseline (T1) hemodynamic data and blood samples, spinal anaesthesia was administered then a pneumatic tourniquet was inflated, and the tested drug was infused intravenously.

Ketamin group (group I) 0.5 mg/kg/hr of ketamine is infused.

Propofol group (group II) 2 mg/kg/hr of propofol is infused.

Ketamin Propofol group (group III) a mixture of 0.5 mg/kg/hr of ketamine & 2 mg/kg/hr of propofol is infused.

Sample Size

G*Power software version 3.1.0 was used to calculate the sample size. According to Statistical Analysis, a sample size of 20 cases in each study group will be required to detect an effect size f of 0.4 in the primary outcome of interest, assuming a type I error of 0.05 and 80% power.

- **Exclusion criteria**

Morbid obesity cases (body mass index >35 kg/m2), cases with coagulation disorders or treatment with anticoagulants, patients with allergy to local anesthetics, propofol or ketamin, bone deformities such as scoliosis & rheumatoid arthritis, antioxidant drug intake, infection or Patient with chronic disease &Cardiac , liver, or renal diseases.

- **Preoperative investigation:**

All study participants were required to undergo:Complete medical history taking, Clinical examination, chest and heart auscultation and Routine laboratory investigation as CBC, kidney function, liver function, Serum glucos level and serum electrolytes.

Intraoperative monitoring includes: Non-invasive blood pressure monitoring, pulse oximetry, and a three-lead electrocardiogram.

- **Study Design:**

All groups' starting values for hemodynamic parameters were recorded. Blood was obtained for the purpose of determining preischemia levels (T1).

ABG samples are drawn for assessment of the preischemia level of po2, pH and Pco2, 2ml of arterial blood was drawn in a heparinized disposable syring for arterial blood gas analysis.

Biochemical studies: Serum MDA level and Serum Catalase level.

The following times saw the collection of blood samples: Before induction of anesthesia (T1), Five minutes after tourniquet deflation (T2), 30 minutes after tourniquet deflation (T3)

STATISTICAL ANALYSIS:

The data was processed with SPSS 20.0 (Statistical Package for the Social Sciences). Mean & standard deviation were utilized to summarise quantitative data (SD). Percentages & frequencies were used to represent qualitative data.

Here are the results of the tests conducted:

When matching more than 2 means, use a one-way ANOVA. When comparing several variables at once, we employed the Post Hoc test. When matching percentages of 2 qualitative variables, the Chi-square (X2) test of significance was utilized. The significance level for the probability (P-value) was set at 0.05. A very significant P-value was defined as less than 0.001. If the p-value was higher than 0.05, it was not believed to be statistically significant.

3. RESULTS

Table (1): Comparison among groups as regard to demographic data

Demographic Data	Group I	Group II	Group III	F/x^2*	p-value
Age (years)					
Mean±SD	34.30±5.49	33.70±4.34	35.15±6.21	0.364	0.697
Range	25-47	28-43	25-47		
Gender [No. (%)]					
Male	11 (55%)	12 (60%)	9 (45%)	0.938*	0.626
Female	9 (45%)	8 (40%)	11 (55%)		
Height					
Mean±SD	170.20±6.03	171.55±6.18	171.35±6.18		
Range	161-181	161-181	161-181	0.282	0.755
Weight					
Mean±SD	75.80±5.59	75.80±6.14	76.95±6.04	0.251	0.779
Range	67-86	67-88	67-91		
Duration					
Mean±SD	39.25±6.20	40.30±5.60	39.20±5.43	0.233	0.793
Range	28-50	30-50	30-48		
Tourniquet					
Mean±SD	44.40±6.09	45.05±5.92	44.15±5.24	0.130	0.878
Range	35-57	36-57	36-53		
ASA				0.0000	1.000
I	10 (50%)	10 (50%)	10 (50%)		
II	10 (50%)	10 (50%)	10 (50%)		

There was no statistically significant difference amongst groups as regared to demographic data (P-valu>0.05) age, gender, mass, height, period of surgery, tourniquet period or ASA physical status.

Table (2): Comparison between groups as regard HR

HR	Group I	Group II	Group III	F-test	p-value
10 min					
Mean±SD	71.85±7.27	71.60±7.49	71.25±6.70	0.035	0.965
Range	61-85	60-85	59-84		
20 min					
Mean±SD	70.95±7.14	72.70±7.88	67.55±7.04	2.530	0.089
Range	60-89	61-86	55-79		
30 min					
Mean±SD	70.95±5.27	71.30±6.42	66.05±6.70	2.539	0.095
Range	60-84	61-83	53-77		
40 min					
Mean±SD	71.50±7.11	70.90±6.44	66.70±5.87	2.126	0.072
Range	61-85	Z60-83	58-75		
50 min					
Mean±SD	69.85±7.10	72.20±6.43	67.35±6.30	2.684	0.077
Range	59-89	63-89	59-78		
60 min					
Mean±SD	70.35±6.60	70.70±6.04	67.05±5.24	2.265	0.113
Range	58-84	62-84	58-77		
70 min					
Mean±SD	69.10±5.81	69.25±5.94	68.40±5.40	0.126	0.882
Range	57-80	60-83	60-77		

There was no statistically significant difference between groups concerning HR during surgery (P-Value>0.05) at all times of recording.

Table (3): Comparison between groups according to catalase

Catalase	Group I	Group II	Group III	F-test	p-value
T1					
Mean±SD	0.85±0.07	0.85±0.06	0.83±0.08	1.529	0.226
Range	0.71-0.96	0.75-0.97	0.71-0.97		
P_1	0.340				
P_2			0.437		
P_3		0.086			
T2					
Mean±SD	0.90±0.07	0.89±0.06	0.88±0.08	3.532	**0.037**
Range	0.75-1	0.78-0.99	0.73-0.99		
P_1	0.722				
P_2			0.049		
P_3		**0.031**			
T3					
Mean±SD	0.93±0.07	0.92±0.06	0.90±0.08	5.352	**0.007**
Range	0.79-1.04	0.8-1.01	0.75-1		
P_1	0.722				
P_2			**0.004**		
P_3		**0.011**			

As regard to catalase level in all groups there was highly significant increase of catalase level in T2 compared to T1 and there was highly significant increase in T3 compared to T1 and T2. There was significant increase from base line value and all remaining times in all groups, however the rise was more recorded in group III matched to both group Iand II which was also higher than group I

Table (4): In group comparision of catalase level at all measured times

Catalase	Group III *Mean±SD*	Group II *Mean±SD*	Group I *Mean±SD*
T1	0.85±0.07	0.87±0.06	0.83±0.08
T2	0.89±0.07	0.90±0.06	0.85±0.08
T3	0.93±0.07	0.92±0.06	0.86±0.08
Diff. 1 vs. 2 (p)	-0.040 (p<0.001)	-0.027 (p<0.001)	-0.016 (p<0.001)
Diff. 1 vs. 3 (p)	-0.080 (p<0.001)	-0.051 (p<0.001)	-0.031 (p<0.001)
Diff. 2 vs. 3 (p)	-0.040 (p<0.001)	-0.024 (p<0.001)	-0.015 (p<0.001)

There is gradual increase in catalase activity in all groups from T1 to T3 but this increase is more significant in group III than group II & the lowest increase was in group I.

Table (5): Comparison among groups according malondialdhyed.

Malondialdhyed	Group I	Group II	Group III	F-test	p-value
T1					
Mean±SD	108.95±10.08	110.35±10.76	108.75±10.24	0.141	0.868
Range	90-126	90-127	91-125		
P_1	0.671				
P_2		0.952			
P_3			0.627		
T2					
Mean±SD	105.15±9.96	104.50±10.64	98.15±10.06	3.861	**0.025**
Range	87-122	85-121	80-115		
P_1	0.841				
P_2		**0.035**			
P_3			0.054		
T3					
Mean±SD	101.80±9.97	99.05±10.69	87.90±9.95	10.399	**<0.001**
Range	84-119	79-116	71-104		
P_1	0.398				
P_2		**<0.001**			
P_3			**0.001**		

There was significant decrease among base line value and all remaining times in all groups, but the decrease is more recorded in group III in comparison to both group I and II, which was also (groupII) lower than group I

Table (6): Comparison among groups according RSS

RSS	Group I	Group II	Group III	t-test	p-value
Mean±SD	2.40±0.50	2.60±0.75	3.00±0.92	3.367	**0.041**
Range	2-3	2-4	2-5		

There was statistically significant difference across groups per the RSS as (p-value <0.05) there was asignificant higher rise of sedation level in group III than group II at which patients were also more sedated than patients in group I, as RSS was 2.40±0.50 in group I, 2.60±0.75 in group II & 3.00±0.92 in group III.

Table (7): Comparison between groups according to ABG I.

ABG I	Group I	Group II	Group III	F-test	p-value
PH					
Mean±SD	7.41±0.02	7.41±0.03	7.41±0.02	0.011	0.989
Range	7.37-7.45	7.37-7.45	7.37-7.45		
PCO2					
Mean±SD	38.90±2.36	39.05±2.33	39.00±2.27	0.022	0.979
Range	35-42	35-42	36-42		
HCO3					
Mean±SD	22.70±1.89	22.80±1.85	22.70±1.89	0.019	0.981
Range	20-26	20-26	20-26		

There was no statistically significant difference between groups according ABG I after tourniquet deflation.

Table (8): Comparison between groups according to ABG II

ABG II	Group I	Group II	Group III	F-test	p-value
PH					
Mean±SD	7.38±0.02	7.38±0.02	7.38±0.02	0.005	0.995
Range	7.35-7.4	7.35-7.4	7.35-7.4		
PCO2					
Mean±SD	42.45±1.85	42.60±1.93	42.55±1.93	0.032	0.968
Range	40-45	40-45	40-45		
HCO3					
Mean±SD	19.90±1.52	20.00±1.49	20.00±1.45	0.030	0.970
Range	18-22	18-22	18-22		

There was no statistically significant difference between groups according ABG II after tourniquet deflation.

4. DISCUSSION

Tissue damage is caused by lipid peroxidation during prolonged ischemia due to tourniquet inflation and following reperfusion. because of the lack of oxygen, the membranes of cells become compromised and die. Ca and phospholipid A2 are released and polyunsaturated fatty acids and free radicals are formed when the membrane integrity is broken.

The reaction of free radicals with oxygen during reperfusion results in lipid peroxidation, which in turn enhances membrane permeability & drives leucocyte chemotaxis. Therefore, cells may emit oxygen-derived free radicals and proteolytic enzymes, which cause damage to the DNA, proteins, & lipids contained inside the cells. As a result, byproducts such as 8-hydroxydeoxyguanosine, carbonyls, methioninsulphoxide, & malondialdehyde (MDA) are formed from the oxidation of amino acids.

With the release of a tourniquet, there is an immediate and significant rise in reactive oxygen species (ROS), which initiates oxidative damage.6

In this study, we looked at how tourniquet-induced ischemia-reperfusion damage was affected by low-dose sedation with Ketamine, propofol, or a combination of the two during arthroscopic knee surgery under spinal anaesthesia.

There was no statistically significant difference (p-value > 0.05) amid the 3 groups in terms of hemodynamic measures, including heart rate & blood pressure, in the present research.

MDA level is increased during the ischamic period which is antagonized by both ketamine and propofol infusion. About 5 minutes into the ischemia reperfusion phase and likewise at thirty min after the tourniquet is removed, MDA levels are lowered in all groups, although group III is more affected than group II or group I.

During the ischemia phase, giving ketamine and propofol together greatly reduced MDA levels.**Gogus et al. (2014)** found no significant changes among groups in terms of hemodynamic parameters (P-value > 0.05), lending credence to the findings of the current investigation.

At T1, which represents the starting point, the current study discovered no statistically significant differences in MDA rankings among the three groups. However, at T2, which is five minutes after the tourniquet was released, there was a statistically significant difference. The levels of T3 are significantly different between the groups after 30 minutes after the tourniquet is removed. At T2 level, there is a significant difference amongst groups I and III (P-value 0.035), and at the T3 level, the difference is extremely significant (P-value 0.001). A comparison of the P values for T2 and T3 reveals a statistically significant distinction between levels II and III (0.054 and 0.001 respectively). At T2 and T3, there is no discernible difference between I and II

Furthermore, **KoGucu et al.**[1] found that Total intravenous anesthetic group (Group T) plasma MDA was significantly that of the sham group (Group S) from 30 minutes after tourniquet augmentation to 2 hours after leak. In arthroscopic knee surgery, this study compared the effects of TIVA with propofol as an anesthetic strategy to those of Group S (30 minutes after tourniquet inflation to 6 hours after release) and found that TIVA with propofol may prevent the increase in plasma quantity of MDA as a tourniquet-related ischemia-reperfusion marker.

These finding are in agreement with those of **Gogus et al.**[6]who investigated the impacts of low-dose ketamine and propofol on tourniquet-induced ischemia-reperfusion injury in patients undergoing arthroscopic knee surgery and showed that MDA ranks were lower in Group I (ketamine and propofol group) than Group II (control group) during T2 (control group which receivedsaline infusion). In T3 and T4, there was no significant difference among the groups' MDA levels.

Controlling for the effects of peripheral nerve blocks, **Budi'c et al.**[7] matched the TIVA and inhalation groups to a third group. At 20 minutes after tourniquet release, the plasma MDA concentration in group S (Inhalation group) was significantly higher than in groups T (TIVA group) and R. (regional anathesia group)

To help patients relax during arthroscopic knee surgery including the use of a pneumatic tourniquet, **Saricaoglu et al.**[8] administered a modest dosage of ketamine infusion (0.5 mg/kg/hr). By comparing the ketamine group to the control group, it was shown that tissue levels of malondialdehyde (MDA) and hypoxanthine (HX), markers of ischemia-reperfusion damage, were much lower in the ketamine group.

For their study titled "The Effects of Dexmedetomidine on Ischemia Reperfusion Damage in Patients Undergoing Arthroscopy Under Spinal Anesthesia," **Koruk et al.**[9] investigated the impacts of dexmedetomidine and ketamine infusion on plasma MDA generation during the early reperfusion period.

It was found in the current study that catalase (CAT) activity was highest during the ischemia phase, at 5 minutes post-reperfusion, and again at 30 minutes post-reperfusion. However, Group III is considerably more active than Groups II and I.

Consistency between the current study's findings and those of the impact of a minor-dose ketamine-propofol mixture on tourniquet-induced ischemia reperfusion damage during arthroscopic knee surgery by **Gogus et al.**[6]

During the ischemic and reperfused stages, Group II's (Ketamin, propofol) catalase activity was higher.: **Turan et al.**[10] Patients undergoing halothane-maintained general anaesthesia had significantly reduced CAT activity 5 and 20 min ATR, and this effect was hypothesised to be related to increased H202 Production.

However, in an experimental model of skeletal muscle IR injuries, **Bosco et al.**[11] found that plasma CAT activity increased after reperfusion. Anesthetic doses of thiopental, etomidate, ketamine, and propofol all resulted in the same level of CAT activity in rats.

The present research found statistically significant differences amongst the three groups on the Ramssy sedation score (RSS). The RSS ranged from 2-4 in Group I, 2-4 in Group II, and 2-5 in Group III for the third group that received both ketamine and propofol.

A different study looked at the impacts of ketamine and dexmedetomidine on ischemia reperfusion injury in cases undergoing spinal arthroscopy, conducted by **Koruk et al.**[9] RSS values were not significantly different amongst groups.

By analysing arterial blood gases, the existing research found no statistically significant difference among groups according to ABGI prior to surgery and no statistically significant difference amongst groups as regard ABG II after tourniquet deflation.

Lee et al.[12] conducted research on the impact of high doses of vitamin C on oxidative stress and myocardial enzyme release during tourniquet-induced ischaemia and reperfusion injury. Following a total knee replacement, the VC group had significantly higher PaO2 and SaO2 levels than the control group 5 minutes after the first tourniquet was punctured & 5 minutes after the second tourniquet was deflated.

PaO2 and SaO2 levels in the control group were significantly lower 5 minutes after the first tourniquet was deflated, and again 5 and 20 minutes after the second tourniquet was deflated, compared to 20 minutes after anaesthesia induction, but there were no significant within-group differences in the VC group at any time point.

When comparing PaCO2 20 minutes after anesthesia was administered or before tourniquet inflation, there were no significant changes between the two groups. However, PaCO2 was considerably greater 5 minutes after the first and second tourniquets were depressed. Tourniquet inflation was said to create anaerobic metabolism and acidosis in the ischemic area, and increased concentrations of hydrogen ions were said to make it easier for carbon dioxide to be produced at the site of ischaemia.

It's possible that the significantly longer tourniquet length in our study resulted in the discharge of hypercapnoeic venous blood from ischemic regions into the systemic circulation and an increase in blood carbon dioxide concentrations after tourniquet deflation.

5. REFERENCES

1. Koşucu M, Coşkun İ, Eroglu A, et al. The effects of spinal, inhalation, and total intravenous anesthetic techniques on ischemia-reperfusion injury in arthroscopic knee surgery. BioMed Research International. 2014;2014
2. Chen S, Hua F, Lu J, et al. Effect of dexmedetomidine on myocardial ischemia-reperfusion injury. International Journal of Clinical and Experimental Medicine. 2015;8(11):21166.
3. Xingwei X, Xin G, Peng Z, et al. Low-dose ketamine pretreatment reduces oxidative damage and inflammatory response following CO2 pneumoperitoneum in rats. Clinical and Investigative Medicine. 2014:E124-E130.
4. Hsiao H-T, Wu H, Huang P-C, Tsai Y-C, Liu Y-C. The effect of propofol and sevoflurane on antioxidants and proinflammatory cytokines in a porcine ischemia–reperfusion model. Acta AnaesthesiologicaTaiwanica. 2016;54(1):6-10.
5. Eroglu A. The effect of intravenous anesthetics on ischemia-reperfusion injury. BioMed research international. 2014;2014
6. Gogus N, Akan B, Bayrakci S, Girgin G, Baydar M. The effects of a small-dose ketamine-propofol combination on tourniquet-induced ischemia-reperfusion injury during arthroscopic knee surgery. Journal of clinical anesthesia. 2014;26(1):46-51.

7. BUDIC I, PAVLOVIC D, KOCIC G, et al. Biomarkers of oxidative stress and endothelial dysfunction after tourniquet release in children. Physiological Research. 2011;
8. Saricaoglu F, Dal D, Salman AE, Doral MN, Klnç K, Aypar Ü. Ketamine sedation during spinal anesthesia for arthroscopic knee surgery reduced the ischemia-reperfusion injury markers. Anesthesia & Analgesia. 2005;101(3):904-909.
9. Koruk S, Mizrak A, Kaya R, et al. The effects of dexmedetomidine on ischemia reperfusion injury in patients undergoing arthroscopy under spinal anesthesia. The Eurasian Journal of Medicine. 2010;42(3):137.
10. Turan R, Yagmurdur H, Kavutcu M, Dikmen B. Propofol and tourniquet induced ischaemiareperfusion injury in lower extremity operations. European Journal of Anaesthesiology. 2007;24(2):185-189.
11. Bosco G, Yang Zj, Nandi J, Wang J, Chen C, Camporesi EM. Effects of hyperbaric oxygen on glucose, lactate, glycerol and anti- oxidant enzymes in the skeletal muscle of rats during ischaemia and reperfusion. Clinical and experimental pharmacology and physiology. 2007;34(1- 2):70-76.
12. Lee J, Kim C, Chung M. Effect of high-dose vitamin C on oxygen free radical production and myocardial enzyme after tourniquet ischaemia–reperfusion injury during bilateral total knee replacement. Journal of International Medical Research. 2010;38(4):1519-1529.