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Impact of oxidative stress on early postoperative knee function and muscle injury biochemical markers: Is it possible to create an ischemic preconditioning effect in sequential ischemic surgical procedures?

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Objective: Simultaneous bilateral total knee arthroplasty (TKA) with prolonged tourniquet time has the potential to trigger ischemia-reperfusion injury, which can adversely affect knee function. Studies suggest that the magnitude of injury is less if it occurs following an ischemic event which takes place in another part of the body, known as ischemic preconditioning (IPC). The purpose of this study was to investigate the impact of oxidative stress on muscle injury and knee function and to elucidate if potential IPC effect can attenuate ischemia-reperfusion injury metabolites and prevent poor functional outcomes in single-stage bilateral TKA.

Methods: Thirty patients who underwent single-stage bilateral TKA under tourniquet were enrolled in the study. All procedures were initiated from the right limb. Upon completion of the procedure, the left tourniquet was inflated 20 minutes after the first tourniquet was deflated. The tourniquet time was noted. Pre- and postoperative levels of malondialdehyde (MDH), creatine kinase (CK), and lactate dehydrogenase (LDH) were evaluated. Knee function was assessed postoperatively at 1 month using WOMAC score.

Results: Postoperative levels of MDH, CK, and LDH were significantly increased in both extremities compared to preoperative levels. Serum MDH, CK, and LDH levels were not found to be correlated with tourniquet time for either extremity. Compared to the left extremity, the right extremity revealed increased postoperative oxidative stress, which was indicated by elevated serum MDH, CK, and LDH levels. Although tourniquet time and postoperative serum MDH, CK, and LDH levels were not found to be correlated with WOMAC index in either knee, the average change in WOMAC score at 1 month postoperatively was found to be higher in the left knee compared to the right.

Conclusion: The biochemical and functional outcomes can be attributed to potential IPC effect. During bilateral TKA, a 20-minute interval between tourniquets can create IPC effect and attenuate the magnitude of ischemia-reperfusion injury, preserving better functional outcomes.

Keywords: Bilateral total knee arthroplasty; ischemia-reperfusion injury; ischemic precondition effect; tourniquet.

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Pneumatic tourniquets are commonly used in the orthopedic field to reduce blood loss and maintain a clear surgical field in limb surgery. Despite their beneficial effects, tourniquet-related nerve paralysis, vascular injury, thromboembolism, compartment syndrome, and limb ischemia–reperfusion injury (IRI) are well defined and continue to be a source of substantial morbidity and mortality in orthopedic surgery.[1,2] Total knee arthroplasty (TKA) is a common ischemic surgical procedure for the treatment of advanced stages of knee degenerative arthritis. Although there has been a tendency in recent years to perform TKA without tourniquet to avoid the complications mentioned above, the vast majority of bilateral total knee replacement surgeries still use ischemic procedures.

Especially in prolonged procedures, IRI has the potential to trigger a systemic inflammatory response and cause multiple organ dysfunctions, including pronounced pulmonary, cardiovascular, and neuromuscular complications.[3,4] Following tourniquet deflation, reperfusion and oxygenation result in the excessive formation of free radicals. The release of these oxygen free radicals induces endothelial dysfunction and the infiltration of activated leukocytes, which play a critical role in systemic injury.[5,6] Reactive oxygen species (ROS) initiate peroxidation of polyunsaturated fatty acids in membrane or plasma lipoproteins, inhibit mitochondrial respiratory chain enzymes, and cause DNA damage in human leukocytes.[7,8] Oxygen free radicals cause transient neutrophil and monocyte activation with enhanced neutrophil transendothelial migration, leading to organ injury as well as apoptosis. Base damage products of this injury are carbonyls, various amino acid modifications such as methionine sulfoxide, and malondialdehyde (MDA). As ROS has a very short half-life and the measurement of free radical activity *in vivo* is complicated and thus not practical, the measurement of the resultant products is preferred. The formation of lipid peroxides following the production of ROS in reperfusion injury can be measured by MDA or by the specific peroxidative lipid product, phosphatidylcholine hydroperoxide. Measurement of these metabolites enables determination of the magnitude of oxidative stress.

Several studies suggest that the magnitude of injury is less if it occurs after an ischemic event which takes place in another part of the body. This condition is known as the ischemic precondition (IPC) effect. Although it is possible that ROS along with prolonged ischemia can indirectly influence postoperative outcomes by adversely effecting fibroblast proliferation and the vascular and peripheral neuromuscular systems, we hypothesize that releasing the first tourniquet following an ischemic TKA procedure may initiate an IPC effect for the sequential second procedure and attenuate the cytotoxic effects of ROS.

The aim of this study is to investigate if a potential IPC effect can attenuate the production of IRI metabolites and prevent poor functional outcomes in single-stage bilateral TKA.

Patients and methods

Demographic data of 30 patients who underwent bilateral TKA under ischemic tourniquet is given in Table 1. A single surgeon using the same technique and implant design conducted all procedures. A 22G catheter was inserted with heparin lock in the right arm and dorsum of each foot, with an intravenous line for preoperative blood sampling. Sterile tourniquets were applied at a pressure twice the systolic arterial pressure, and tourniquet time was noted separately for both extremities. The procedure was initiated with the right knee. The right limb tourniquet was inflated first and released only after wound closure and compressive dressing upon completion of the procedure. The second tourniquet on the left limb was inflated 20 minutes after the first tourniquet was deflated. Five ml of venous blood samples were obtained preoperatively from an antecubital intravenous line and postoperatively from each lower extremity 20 minutes following the release of each tourniquet. The blood were centrifuged at 1.000 rpm for 10 min, and the serum was stored at –80°C for later measurement. Pre- and postoperative levels of MDA, creatine kinase (CK), and lactate dehydrogenase

Table 1. Demographic data and tourniquet time of patients.

	n	Mean±SD	Minimum	Maximum
Age (years)	30	64.2±5.22	51	77
Weight (kg)	30	76±6.9	60	112
Height (cm)	30	1.59±0.07	1.49	1.75
Body mass index (kg/m²)	30	30.66±2.81	28	35
Right tourniquet time (minutes±SD)	30	60.9±5.2	52	66
Left tourniquet time (minutes±SD)	30	61.9±5.1	53	66

Table 2. Preoperative and postoperative WOMAC index scores for each knee.

	Preop R knee	Postop R knee	Preop L knee	Postop L knee
1	61	43	65	41
2	67	46	58	41
3	67	48	75	45
4	63	45	68	39
5	65	46	73	42
6	69	53	71	44
7	71	51	66	37
8	68	47	62	43
9	66	46	66	47
10	68	49	59	41
11	65	44	61	42
12	65	46	63	44
13	65	46	65	37
14	67	49	57	39
15	65	47	65	39
16	67	49	74	42
18	63	44	63	41
19	65	48	64	45
20	70	51	60	40
21	64	46	67	44
22	65	47	71	46
23	67	47	61	40
24	63	44	66	39
25	63	42	65	44
26	64	42	56	39
27	66	45	67	44
28	63	48	63	42
29	64	45	67	44
30	70	52	73	46

R: Right; L: Left; Preop: Preoperative; Postop: Postoperative.

(LDH) were evaluated. Serum MDA levels were measured in duplicate aliquots, using a human enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (Oxiselect™, CellBiolabs Inc, San Diego, CA, USA). BSA standards or protein samples (10 μg/mL) were adsorbed into a 96-well plate for 2 hours at 37°C. The MDA-protein adducts present in the sample or standard were probed with an anti-MDA antibody, followed by a horseradish peroxidase-conjugated secondary antibody. The ELISA wash steps were performed with ELx ELISA washer. Following the substrate and stop solution application, the quantity of MDA adduct was measured spectrophotometrically at 450 nm (620 nm as optional reference wave length) with an ELISA reader (ELx80™, BioTek, Winooski, VT, USA). The MDA-protein adducts content in an unknown sample was determined by comparison against a standard curve prepared from predetermined MDA-bovine serum albumin standards. The results were expressed as pmol/mg. Serum CK and LDH levels were measured. Creatine phosphokinase (U/L) was measured with Abbott original kit (Abbott Laboratories, Abbott Park, IL, USA). CK was measured with N-acetyl cysteine (NAC). Activated LDH (U/L) was measured with lactate to pyruvate method by Abbott ARCHITECT c16000 analyzer (Abbott Laboratories, Abbott Park, IL, USA). Pre- and postoperative 1 month TKA was assessed using the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) for each knee (Table 2).

Statistical comparisons were generated using Statistical package for Social Sciences version 11 for Windows (SPSS Inc., Chicago, IL, USA). All data are expressed as mean±SD. P values >0.05 were considered statistically significant.

Results

Postoperative levels of MDA, CK, and LDH were significantly increased in both the right and left knees compared to preoperative levels (Table 3). Compared to the left knee, the right knee revealed increased postoperative oxidative stress and muscle injury biochemical parameters, as indicated by elevated serum MDA, CK,

Table 3. Preoperative and postoperative levels of serum MDA, CK, and LDH in both right and left knees.

	n	Mean	Minimum	Maximum
Preop malondialdehyde	30	23.15±12.9	10.38	53.8
Malondialdehyde-Left	30	93.73±35.5	42.3	173.23
Malondialdehyde-Right	30	96.41±47.43	40.12	189.42
Preop lactate dehydrogenase	30	194.80±80.5	94	377
Lactate dehydrogenase-Left	30	225.8±109.4	79	536
lactate dehydrogenase-Right	30	243.2±68.3	98	445
Preop creatine kinase	30	75±104.1	23	588
Creatine kinase-Right	30	67.12±35.4	22	256
Creatine kinase-Left	30	63.15±26,9	19	193

Table 4. Duration of ischemia for both left and right knees.

Patient	Right tourniquet (minutes)	Left tourniquet (minutes)
1	65	66
2	61	65
3	58	66
4	53	65
5	54	64
6	64	60
7	64	66
8	66	53
9	56	53
10	54	58
11	66	53
12	65	66
13	65	66
14	65	54
15	64	55
16	63	57
17	66	66
18	64	54
19	66	66
20	58	65
21	65	66
22	66	66
23	53	65
24	56	65
25	64	66
26	65	65
27	54	66
28	52	59
29	63	66
30	52	56

and LDH levels (Figures 1–3). Duration of the tourniquet for each knee was recorded, and the critical time of 120 minutes was not exceeded, as it would compromise the oxidative stress biochemical parameters and related potential IPC effect (Table 4). Serum MDA, CK, and LDH levels were not correlated with tourniquet time for either lower extremity.

Although it was not statistically significant, the average change in WOMAC index score at 1 month postoperatively was found to be higher in the left knee (23.2±4.46) compared to the right (18.9±1.54). Left knee WOMAC index score was not correlated with mean left tourniquet time or lower extremity postoperative serum MDA, CK, and LDH levels. Similar to the left knee, right knee WOMAC index score was not correlated with mean right tourniquet time or right lower extremity postoperative serum MDA, CK, and LDH levels.

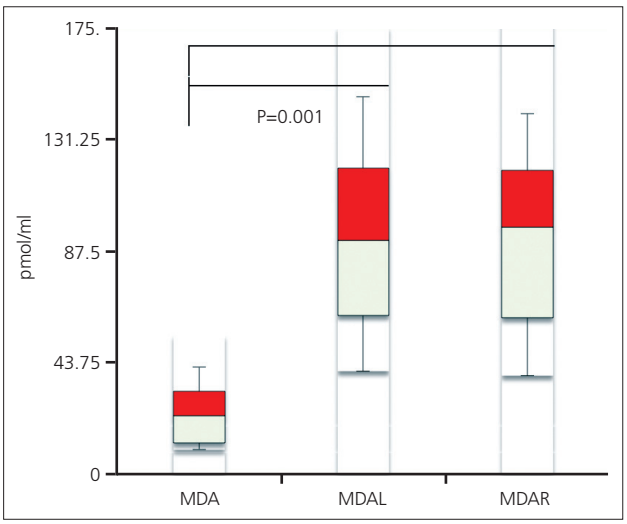

Fig. 1. Preoperative serum MDA levels and postoperative levels of serum MDA after release of right and left knee tourniquets. [Color figures can be viewed in the online issue, which is available at www.aott.org.tr]

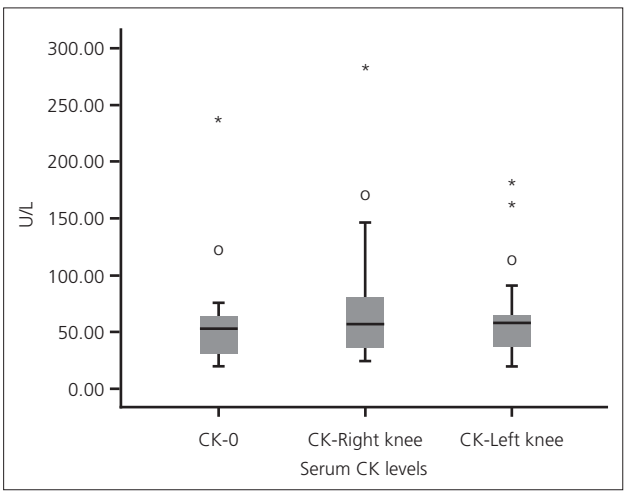

Fig. 2. Preoperative serum CK levels and postoperative levels of serum CK after release of right and left knee tourniquets.

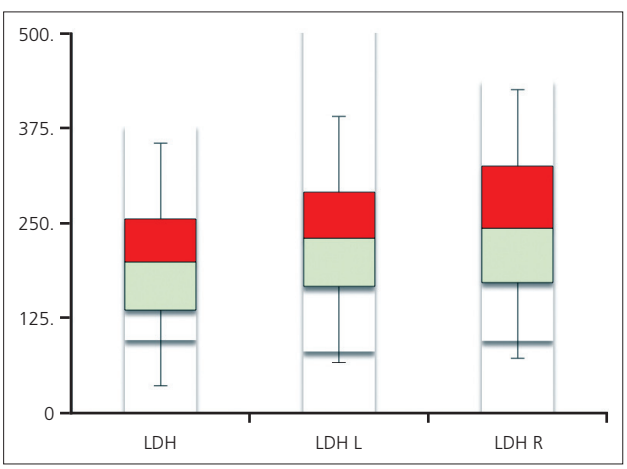

Fig. 3. Preoperative serum LDH levels and postoperative serum LDH levels after release of right and left knee tourniquets.

Discussion

Although there has been a tendency to perform TKA without a tourniquet in recent years, the vast majority of this procedure is still performed under ischemic conditions using a pneumatic tourniquet. Success in an uncomplicated TKA is directly related to adequate postoperative neuromuscular function and wound healing. Mohler et al. reported an increase in the prevalence of electromyographic abnormalities.[9] They concluded that in upper limb ischemic surgery, tourniquet-induced neuropathy played a causal role in impaired rehabilitation. Electromyographic abnormalities were correlated with tourniquet time, and evidence of denervation typically lasted from 2–6 months. A review of rabbit and clinical experiments revealed that tourniquet-induced neuromuscular injury.[10] In the current study, LDH and CK levels were found to be elevated in the lower right extremities compared to the lower left extremities, indicating oxidative stress-related muscle injury in a dose-dependent manner.

In order to avoid these tourniquet-related comorbidities, preventive measures such as use of various antioxidant medications (vitamin C, taurine, probucol), pravastatin, propofol, zinc, or reduction of tourniquet time and pressure have shown limited success.[11–16] Koca K et al. concluded that NAC and IPC demonstrated protective effects on the occurrence of oxidative stress resulting from IRI by preventing MDA, superoxide dismutase, and glutathione peroxidase changes in ischemic arthroscopic knee surgery.[17] It is crucial for orthopedic surgeons to understand the mechanism of free oxygen radical production and their effects on ischemic limb surgery. Our main goal is to elucidate the relation between the magnitude of the oxidative stress-related tissue injury and IPC effect, as this correlation can guide us to take preoperative and intraoperative preventive measures and develop specific postoperative treatment modalities.

As an end product of lipid peroxidation, MDA levels have the potential to reflect the magnitude of IRI, and the duration of tourniquet application may influence IRI metabolites; MDA, LDH, and CK production may have a prominent adverse effect on postoperative functional outcomes. Kharbanda et al. concluded that ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans, and Gho et al. concluded transient renal ischemia limits myocardial infarct size in rats.[18,19]

Adverse effects of free oxygen radicals on various tissues and organs have been demonstrated in multiple studies, yet these effects on fibroblasts, myoblasts, and endothelial cells have not been elucidated in a dose-dependent manner. As a consequence of delayed tourniquet time, ROS have been shown to be associated with toxicity on neuromuscular tissue and can initiate apoptosis on the molecular level. The mechanism of this injury in the orthopedic field can be described as reperfusion and oxygenation following the deflation of tourniquets, resulting in the excessive formation of ROS. To evaluate ROS production from enzyme, cell, or organ systems, we chose to measure serum-specific lipid peroxidative product MDA, one of the most toxic metabolites created by ROS production.

Proteins, nucleic acids, polyunsaturated fatty acids, and lipids are polysaccharides with greater susceptibility to ROS attack. As a result of excessive ROS production, MDA produced by activated neutrophils has a pivotal role in the damage of endothelial cells.[20–22]

Contrary to the above findings, there is evidence that oxygen free radicals can both stimulate and inhibit proliferation of cultured human fibroblasts and that fibroblasts themselves can release superoxide (O_2-) free radicals in a dose-dependent manner. George et al. concluded that low concentrations of oxygen free radicals stimulate cultured fibroblasts to proliferate and that cultured fibroblasts release their own free radicals.[23]

Similarly, IPC has been reported to have a protective effect on endothelial injury and to inhibit systemic neutrophil activation.[24,25] Sha Y et al. and Murphy et al. studied the genomic response induced by IPC, concluding that IPC was associated with the altered expression of genes involved in neurological system processes and the regulation of neuron apoptosis. Additionally, they found that IPC of the lower limb in TKA patients induced a protective genomic response, which results in the increased expression of immediate early response genes, oxidative stress defense genes, and prosurvival genes. These findings indicate that IPC may be of potential benefit in knee arthroplasty and similar musculoskeletal conditions.[26,27] Though there is evidence that low concentrations of oxygen free radicals and IPC effect can protect ischemic limbs, the gross impact of dose-dependent ROS on vascular and neuromuscular tissues still remains a significant problem.[28] Cheng YJ et al. concluded that production of ROS significantly increased at 5 and 20 minutes after release of the first tourniquet and at 5 minutes after release of the second tourniquet; however, production of ROS returned to normal levels at 20 minutes after the second reperfusion during bilateral TKA.[29] In the current study, although tourniquet times were similar for both knees, serum MDA, CK, and LDH levels were found to be elevated in the right knee compared to left. This data concludes that there may be a preventive effect of ischemic precondition with the release of the

first tourniquet and this effect can attenuate the resultant ischemia-reperfusion injury of the contralateral limb, as the release of the second tourniquet did not produce the expected increase in free radicals.

Although various medications such as systemic administration of taurine, vitamin C, and probucol have shown a degree of beneficial effects as well as attenuating ROS effects, none of these methods have been proven to eradicate IRI.

Despite the IPC phenomenon that occurs during bilateral TKA with sequential tourniquet release, significant markers of excessive ROS production (MDA) and muscle injury biomarkers (CK, LDH) can be detected in extremities with prolonged tourniquet time, revealing the magnitude of reperfusion injury. In the current study, these markers were found to be higher in the right lower extremity compared to left, but neither showed correlation with tourniquet time. Similarly the relationship between MDA levels and postoperative WOMAC score did not reflect a prominent adverse effect of ROS on knee functions in a dose-dependent manner. Although single-stage TKA with sequential tourniquet release is considered to be a safe procedure in clinical practice, the surgeon must always be aware of the potential effects of ROS that are released abruptly following tourniquet deflation.

Despite the debate surrounding the protective effects of IPC against IRI, in this single-stage bilateral TKA study, the resultant biochemical and clinical outcomes can be attributed to a potential IPC effect. Supporting our findings, in 2 studies conducted by Memtsoudis et al., although patients with IPC revealed significant decrease regarding postoperative pain, inflammatory markers (interleukin 6, tumor necrosis factor alpha, C-reactive protein, leukocyte count), muscle oxygenation, and length of hospital stay did not differ between IPC and control groups.[30,31]

In clinical practice, during an ischemic bilateral TKA, inflating the second tourniquet 20 minutes after the first tourniquet is deflated can create an IPC effect and attenuate the magnitude of ischemia-reperfusion injury of the contralateral lower limb neuromuscular and vascular systems.

In the future, tourniquet systems designed to monitor physiologic variations of intraoperative serum lipid peroxidation and neuromuscular injury biomarker levels as well as estimate dynamic limb occlusion pressure on the basis of the obtained data can determine safer ischemic orthopedic surgeries

Conflics of Interest: No conflicts declared.

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