Can the arterial clamp method be used safely where a tourniquet cannot be used?

Ozgur Erdogan, Volkan Gürkan, Cavide Sönmez, Tunay Erden, Sezen Atasoy, Fatih Yildiz, Bekir İnan, Adile Adilli

Abstract

Background: Clamp application is safe and widely used in the visceral organs. This raises the question: why not use clamping in orthopaedic, oncological, fracture and revision surgeries of areas where tourniquets are not suitable. This experimental animal study aimed to compare tourniquet and arterial clamp applications with regard to their histological effects and inflammatory responses on a molecular level, on the artery, vein, nerve and muscle tissue.

Methods: Twenty-one rabbits were divided into three groups (group I: proximal femoral artery clamp; group II: proximal thigh tourniquet; and group III: control group). In the clamp group, the common femoral artery was clamped with a microvascular clamp for two hours. In the tourniquet group, a 12-inch cuff was applied to the proximal thigh for two hours at 200 mmHg. The common femoral artery, vein, nerve, rectus femoris and tibialis anterior muscles were excised and analysed in all groups.

Results: Artery and vein endothelial injuries were found in the clamp and tourniquet groups (relative to the control group, $p \leq 0.001$ and $p = 0.007$, respectively). However, no difference was found between the clamp and tourniquet groups regarding vessel wall injury.

Conclusion: We found there were no differences in incidence of vessel, muscle and nerve injury when comparing the tourniquet and clamp applications. For surgical procedures that are unsuited to a tourniquet, arterial clamping can be selected, resulting in close-to-tourniquet vessel injury rates but without tourniquet-related complications.

Department of Orthopaedics, Health Sciences University, Haydarpasa Numune Education and Research Hospital, Istanbul, Turkey
Ozgur Erdogan, MD, drozgurerdogan@gmail.com

Department of Orthopaedics, Faculty of Medicine, Bezmialem University, Istanbul, Turkey
Volkan Gurkan, MD
Fatih Yıldız, MD

Department of Pathology, Faculty of Medicine, Bezmialem University, Istanbul, Turkey
Cavide Sonmez, MD
Adila Adıllı, MD

Department of Orthopaedics, Fulya Hospital, Acibadem University, Istanbul, Turkey
Tunay Erden, MD

Division of Genetics, Department of Medical Biology, Istanbul University Cerrahpasa, Istanbul, Turkey
Sezen Atasoy, MSc

Department of Cardiovascular Surgery, Faculty of Medicine, Bezmialem University, Istanbul, Turkey
Bekir Inan, MD

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Blood loss can occur during any surgical procedure. However, certain types of surgery are associated with higher amounts of blood loss, which require transfusion. Some of these procedures are kidney, hepatic, orthopedic and vascular operations.[1] Orthopaedic surgery, especially malignant tumour surgeries of the extremities, results in severe blood loss, which causes difficulty in dissection of the tumour and neurovascular structures, and consequently prolonged surgical times, excessive blood transfusion and transfusion-related complications.

Tourniquet use solves this problem, however its use becomes impossible if the surgical field is very proximal, where arterial clamp use could be effective. It should be noted that tourniquet use can be dangerous and in some instances may be contraindicated.[2] Also, there is controversy about the appropriate application time and pressure. Moreover, severe neurological and muscular damage related to the use of a tourniquet has been reported.[3]

In radical surgical procedures, arterial clamp application through an additional small incision and vascular dissection, which can quickly close and open the blood supply, may solve these problems. However, orthopaedic surgeons are often unfamiliar with arterial clamp use or they are unsure about its reliability and/or safety. The literature on arterial clamp use in malignant tumour surgeries of the proximal parts of the extremities is lacking.

This study aimed to investigate the effectiveness and safety of arterial clamps in terms of possible damage to the arterial wall. The main question motivating this work was why we do not use the clamp method in orthopedic, oncological procedures in patients where tourniquet application is impossible. The hypothesis was that tourniquet use has less adverse effects on the arterial wall than the direct application of an arterial clamp.

Methods

This animal study proposal was approved by the local ethics committee and local animal experimental ethics committee (HADYEK) of the Bezmialem University, with permit number: 2017/21. According to the guide, Institutional Animal Care and Use Committee (IACUC)[4] for the care and use of laboratory animals, principles and animal rights were protected in this study. A local ethics committee, approved by Bezmialem University Faculty of Medicine Ethics Committe, approved this study: number 2016/154. In the study design, *Guide for the Care*

and Use of Laboratory Animals[5] was used, and two attendant veterinarians controlled all procedures.

The investigators found it helpful to consult with experts regarding statistical analysis for required animal numbers, and database searches to identify potential alternatives to painful or distressing procedures.[6] Retrobulbar injection of no more than 200 µl of injectable anaesthetic solution (ketamine:xylazine) was used, resulting in death within five seconds of cessation of injection,[7] as mentioned among the forms of euthanasia in the *AVMA Guidelines for the Euthanasia of Animals*, 2020 edition.

Twenty-one New Zealand male white rabbits (eight months old, mean weight 3 kg, range 2.6–3.4 kg) were obtained from a private farm, by veterinary faculty authority. The 21 rabbits were divided into three groups: group I received a proximal femoral artery clamp; group II received a proximal thigh tourniquet, and group III was the control group.

The rabbits were prepared in the supine position after anaesthesia using 2 mg/kg of intramuscular diazepam and 40 mg/kg ketamine, and they were draped after shaving and cleaning their skin with betadine. In order not to affect tumour necrosis factor alpha (TNF-α) values, only one limb side was used in all subjects.

In the clamp group, a proximal incision was made anteromedially over the femoral neurovascular margin, and the skin, subcutaneous tissue and deep fascia were incised. After dissecting the muscles and exposing the neurovascular bundle, the femoral artery was dissected and clamped with a microvascular

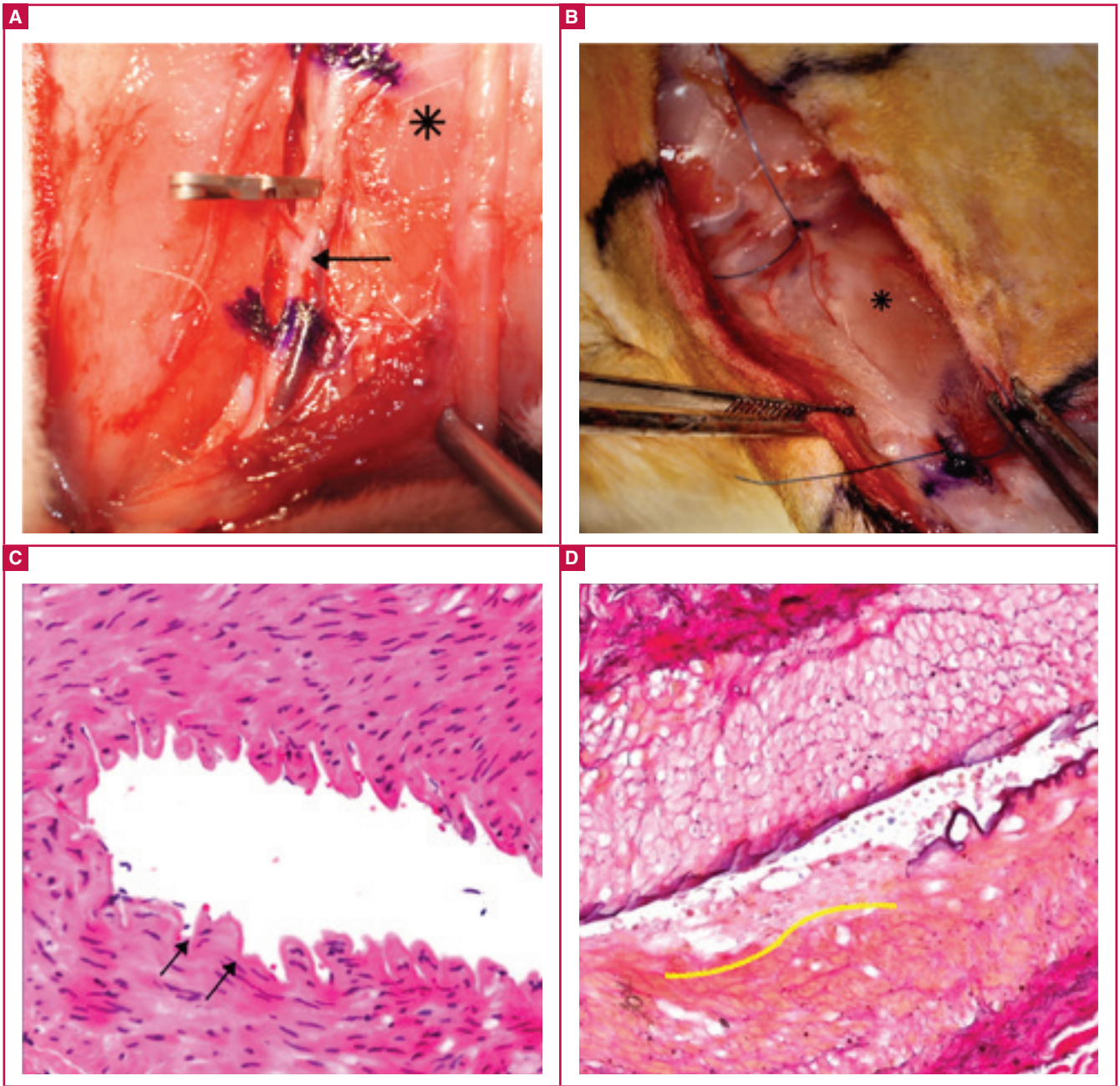

Fig. 1. (A) Clamp: CFA, black arrow: femoral vein, nerve, asterisk: rectus femoris muscle. (B) Asterisk: rectus femoris muscle. (C) Black arrow: areas without endothelial cells (H&E stain, x200). (D) Yellow line: fibrin thrombi (elastin van Gieson stain, x200)

clamp (Biemer vessel clip, 7-mm jaw length, closing force 30 G, MCI-47-104, Medical Care Instruments, Manchester, UK). The area where the clamp was applied was referred to as the 'middle'. The location 1 cm proximally was denoted 'proximal', the location 1 cm distally was denoted 'distal', and both were marked with 4.0 vicryl. At the same level, the femoral vein, femoral nerve and rectus femoris muscle segments were marked in the same fashion. The clamping time was two hours.

At the end of two hours, a longitudinal incision was made on the anterolateral side of the leg and a 5-cm segment of tibialis anterior muscle was excised. From the marked lines, the femoral artery, vein, nerve and rectus femoris muscle were excised. The samples were sent to the pathology laboratory for histological analyses. The animals were euthanised after the procedure.

In the tourniquet group, a 'Blue 12 inch for child' (reference no: 20-54-710, VBM Medizintechnik GmbH, Sulz am Neckar, Germany) tourniquet was used. The standard tourniquet time for all subjects was two hours and the pressure was 200 mmHg.[8] The proximal and distal borders of the cuff were marked with a tissue pen. After two hours, the tibialis anterior muscle sections were excised, as with the clamp group. The tourniquet was released and removed, then the femoral artery, vein, nerve and rectus femoris muscle were excised from the previously marked cuff margins.

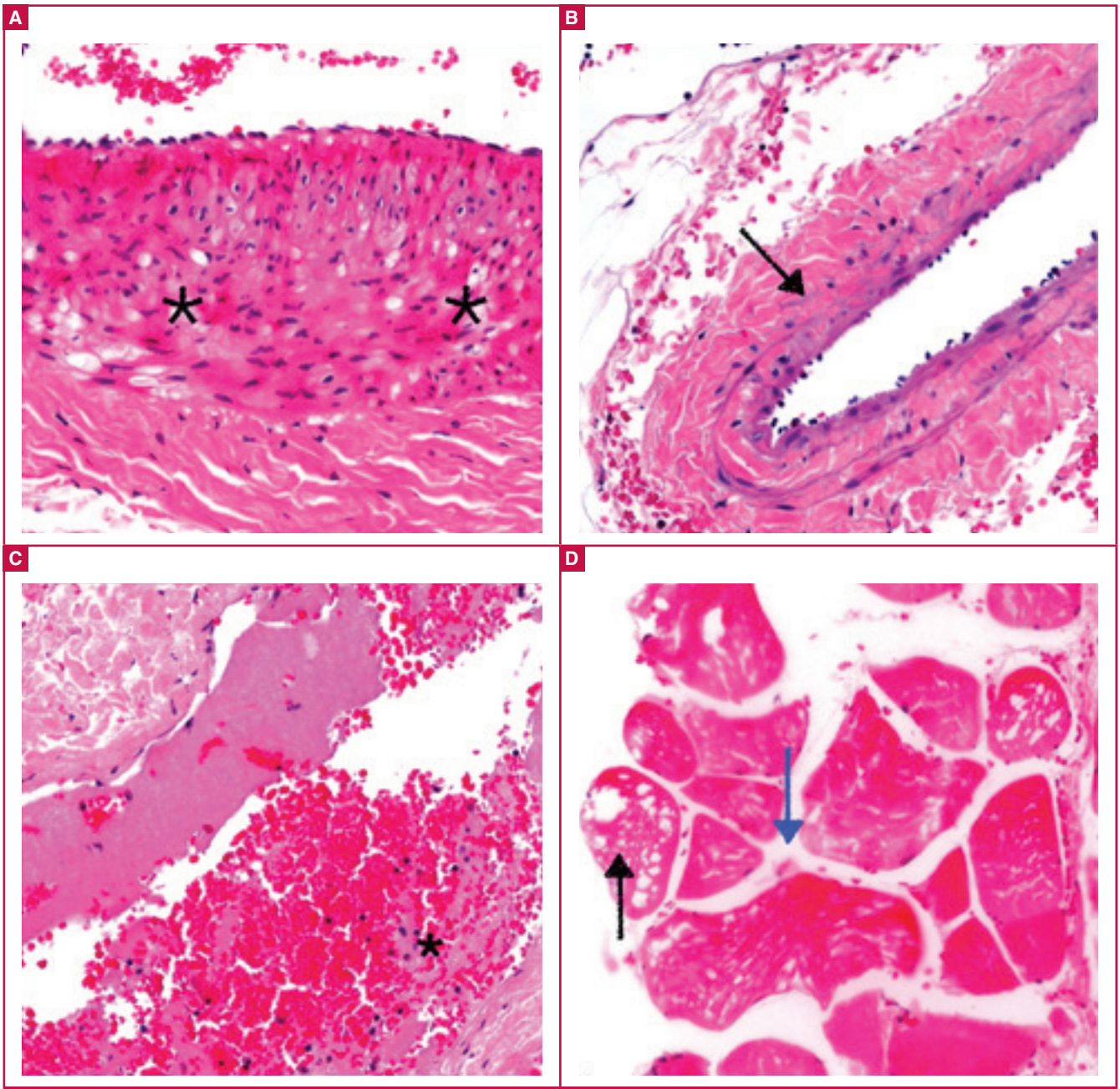

Fig. 2. (A) Black stars: vacuoles (H&E stain, x 200). (B) Severe injury, black arrow: naked areas (H&E stain, x200). (C) Black star: fibrin plaque (H&E stain, x 200). (D) Black arrow: vacuoles with loss of cytoplasmic structures, blue arrow: cytoplasmic eosinophilia.

In the control group, no clamp or tourniquet was used. A longutidinal incision was made on the anterolateral side of the crus muscle. A 5 cm length of tibialis anterior muscle was excised. A longitudinal anteromedial incision was made over the femoral neurovascular bundle. The neurovascular bundle and rectus femoris muscle were marked with 4.0 vicryl suture rope from the proximal and distal borders. Then the bundle and rectus femoris muscle were excised. Histological examination of the common femoral artery, vein, nerve, rectus femoris and tibialis anterior muscle was performed (Figs 1, 2). The animals were euthanised.

Areas with significant histological findings were evaluated. In the clamp group, the arterial specimen was divided into three groups: proximal, the clamp area in the middle, and distal. The aim of this part of the study was to compare the normal tissue in the proximal part of the ischaemic area, the damage to the arterial tissue where the clamp was applied, and the changes due to ischaemia in the distal part of the clamp.

In the tourniquet group, the artery sample was divided into three similar pieces. The aim of this part of the study was to evaluate the effect of pressure differences on the tissue between the proximal and the middle part of the tourniquet cuff, and to examine the effect of ischaemia on the distal side.

In both the tourniquet and the clamp groups, the vein, nerve and muscle tissue were divided into three sections: proximal, middle and distal. In the clamp group, the aim was to evaluate the tissue injury caused by dissection. In the tourniquet group, the aim was to evaluate the effect of pressure difference between the proximal and middle part of the tourniquet cuff, and to examine the effect of ischaemia on the distal side. The tibialis anterior muscle tissue was examined in all three groups to compare the ischaemic injury distal to the extremity. Connective and adipose tissue were not evaluated because no significant light microscopic findings were expected with short-term trauma and hypoxia in these kinds of tissues.

Six slides for the vessels and three slides for the nerves and muscles were obtained per sample. Samples from each group were fixed with 10% neutral-buffered formalin for one day and processed for standard paraffin embedding.

Serial sections (4-µm-thick slices) were cut using a microtome. All of the sections were stained with haematoxylin and eosin (H&E) and vessel sections with elastin van Gieson stain (Ventana) for light microscopic examination (Nikon-Eclipse-80i-DS-Ri1). An automatic device (Ventana, Benchmark XT) was used for histochemical staining. The photographs were captured with a digital camera (Nikon-Eclipse-80i-DS-Ri1). The pathologist, who was blinded to the slide numbers and groups, examined the slides for tissue damage, including sections from the tibialis anterior and rectus femoris muscles, the femoral nerve and the femoral artery and vein.

Degeneration and inflammation were evaluated semi-quantitatively for scoring skeletal muscle injury. Histological findings such as cytoplasmic eosinophilia with loss of cytoplasmic structures, cytoplasmic vacuolation, swelling, loss of striation, fragmantation and rupture were used for scoring muscle degeneration. The score was: 1, findings are mild and focal; 2, moderate and in some areas; 3, severe and common. The following criteria were used for inflammation: score 0, no inflammation; 1, mild inflammation; 2, moderate inflammation, and 3, severe inflammation. The scores were added and the total muscle injury scores were calculated for each group.

Table 1. Score parameters of vessel injury. Total score: 0–3: mild injury, 4–6: moderate injury, 7–9: severe injury

Endothelial damage score		*TMSMV*	
None	1	None	0
Minimal	2	Some cells in deep	1
Moderate desquamation in endothelial cells	3	Consistent	2
Significant desquamation in endothelial cells	4	Superficial + deep	3
Continuity of internal elastic lamina		*Inflammation score*	
Intact	0	None	0
Interruption/fissure	1	Mild	1
Significant fissure	2	Moderate	2
Significant fissure/cavity + thrombus	3	Severe	3

TMSMV: tunica media smooth muscle vacuolation.

Degenerative changes in the peripheral nerve fibres were determined semi-quantitatively according to oedema and axonal degeneration using light microscopy. If the findings were mild, the score was 1; moderate, 2; and severe, 3. Light microscopy gives limited information without electron microscopy findings. Essentially we did not expect serious damage to the nerve as the clamp or tourniquet was applied for a short time only.

Arterial injury was scored using the criteria in Table 1. Endothelial injury was scored using the same method applied for arteries and venules. Venous intimal plaque was also evaluated. Using a quantitative approach, intact endothelium in the 500-µm segment was assessed by number of endothelial cells (NEC) in the arteries and venules. Endothelial damage (score of endothelial ınjury: SEI) was assessed in the venules and arteries. The continuity of the internal elastic lamina (score of lamina elastica interna injury: SLEI) in the arteries was examined, as well as the smooth muscle vacuolation in the medial layer (tunica media smooth muscle vacuolation: TMSMV) in the arteries.

Western blot analysis was used for protein analysis. Tissue samples from the distal part of the clamp group (n = 7), the distal part of the tourniquet group (n = 7) and the control group (n = 7) were snap frozen in liquid nitrogen and stored at –80°C. Being a key regulator for tissue injury TNF-α and also for loading control, beta-actin antibodies were used for analysis and bands were determined using the imaging system (Vilber Fusıon FX, France).

Statistical analysis

Power analysis was done before the experimental set up to determine the number of animals required. The difference between the groups was predicted using Margovsky *et al.* 'area of endothelial damage' values.[9] The effect size (d) was 2.19 in the calculation for obtaining 80% power at the α = 0.05 level. Accordingly, it was determined that there should be at least seven members in each group.

Descriptive statistics were used to define continuous variables (mean, standard deviation, minimum, median, maximum). Comparisons of independent variables with normal distribution were performed using the Student's t-test. Comparisons of two independent and non-normal distributions were performed using the Mann–Whitney U-test. The chi-squared test (or Fisher's exact test at appropriate locations) was used to examine the relationship between categorical variables. The statistical significance level was determined as 0.05. The analysis was performed using MedCalc Statistical Software version 12.7.7

(MedCalc Software BVBA, Ostend, Belgium) and ordinary one-way ANOVA was performed using GraphPad Prism version 7.0c (GraphPad Software, La Jolla California USA).

Results

For the artery, the NEC values of the clamp and tourniquet groups were lower than those of the control group ($p \leq 0.001$, $p = 0.007$, respectively), while the other parameters of the clamp and tourniquet groups were higher than those of the control group (SEI, $p \leq 0.001$ and $p \leq 0.001$; SLEI, $p \leq 0.001$ and $p = 0.004$; TMSMV, $p = 0.004$ and $p = 0.008$; total score, $p \leq 0.001$ and $p \leq 0.001$, respectively). When the clamp and tourniquet groups were compared, no differences were found for all vascular parameters (Fig. 1C, D, Table 2).

For the vein, the NEC values of the clamp and tourniquet groups were lower than those of the control group (both $p \leq 0.001$). The SEI values were higher in the clamp group than in the control group ($p = 0.055$). In the tourniquet group, all values were higher than in the control group ($p = 0.023$) (SLEI, both $p \leq 0.001$). There was no difference between the distribution of plaque in the distal, middle and proximal regions of vessels in the clamp and tourniquet groups (Fisher's exact test, $p > 0.05$) ($p = 0.286$, 0.265, 1.00, respectively). No difference was found between the clamp and tourniquet groups for all parameters.

For nerve and muscle tissue, there were no differences between the groups regarding femoral nerve injury scores, rectus femoris and tibialis anterior degeneration, inflammation and total injury scores ($p = 0.533$, 0.876, 0.604, 0.756, respectively). There were also no differences between the middle regions of vessels of the clamp group and the mean values of the tourniquet group (Mann–Whitney *U*-test, $p > 0.05$).

Protein levels were evaluated by Western blotting. The samples were normalised using beta-actin levels. The bands were analysed by densitometry and normalised using Image J Software (National Institutes of Health, USA). Statistical analysis was carried out by ordinary one-way ANOVA using GraphPad Prism version 7.0c (GraphPad Software, La Jolla California USA) and no statistically significant differences were found between the groups for TNF-α values ($p = 0.1712$) (Fig. 3).

Discussion

To our knowledge, this is the first study comparing tourniquet and arterial clamping in the literature. We found no significant difference between tourniquet and clamp methods regarding histological and inflammatory response in the vessel. Therefore, the clamp method can be used in orthopedic oncological, trauma and revision hip-joint surgeries that are unsuited to a tourniquet. The external iliac artery or axillary artery can be

Table 2. Clamp versus tourniquet versus control group according to injury parameters

Group	*NEC*	*SEI*	*SLEI*	*TMSMV*	*Total score*
Clamp					
Min–max	1–27	1–3	0–1	0–2	1–5
Mean ± SD (median)	14.43 ± 10.6 (18)	1.71 ± 0.95 (1)	0.29 ± 0.49 (0)	1.14 ± 0.69 (1)	3.14 ± 1.46 (3)
Tourniquet					
Min–max	9–30	1–2	0–1	0–2	1–4
Mean ± SD (median)	18 ± 7.55 (15)	1.57 ± 0.53 (2)	0.43 ± 0.53 (0)	0.43 ± 0.79 (0)	2.43 ± 1.4 (3)
Control					
Min–max	20–33	0–0	0–0	0–2	0–2
Mean ± SD (median)	24.57 ± 4.2 (25)	0 ± 0 (0)	0 ± 0 (0)	0.43 ± 0.79 (0)	0.43 ± 0.79 (0)
p-value	0.099	0.001*	0.174	0.103	0.004*

*Kruskal–Wallis test, $p < 0.05$.
NEC: number of endothelial cells; SEI: score of endothelial injury; SLEI: score of lamina elastica interna injury; TMSMV: tunica media smooth muscle vacuolation.

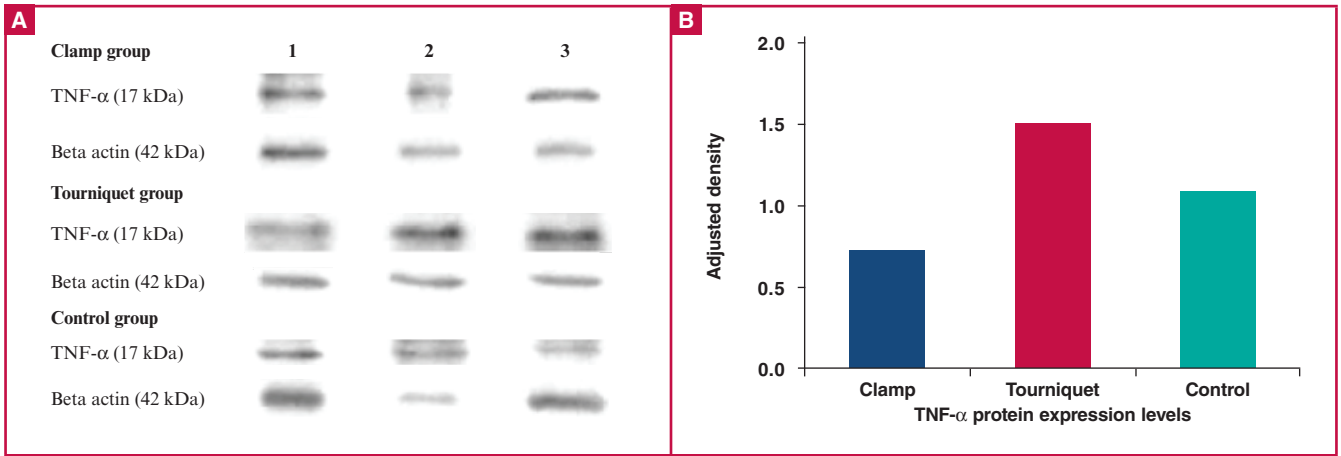

Fig. 3. (A) Representative immunoblotting images of TNF-α protein expression were examined by Western blotting. Beta actin was used as equal loading control in analysis. (B) Densitometric analysis of Western blot results.

clamped by a vascular surgeon at the beginning of a revision joint surgery or resection of a proximal limb tumour. With careful vessel dissection and the minimum pressure required for occlusion, clamp-related complications can be avoided.

Interrupting blood flow using an arterial clamp or a tourniquet is associated with haemodynamic changes and leads to inflammation, which triggers pathophysiological processes.[10] Zammert *et al*. reported that with arterial clamp application, TNF-α played a crucial role in haemodynamic changes and was associated with tissue injury.[11] Although the behaviour of endotoxins after clamping is unclear, Caty *et al*. showed that TNF-α was involved in the initiation of injury.[12]

TNF-α has a central role in initiating an inflammatory response by engaging multiple pathways, especially mitogen-activated protein (MAP) kinases and caspase proteases. MAP kinases increase TNF-α expression and induce a secondary response.[13] Interactions between MAP kinases and TNF-α contribute significantly to tissue regulation for cell response in damage and cellular homeostasis.[14] MAP kinases, also known as stress-response kinases, are triggered by environmental stressors. Mechanical damage of tissue activates the MAP kinase (JNK and p38) pathway stimulated by TNF-α, and activated MAP kinases alter physiological responses in the process of various diseases.[15,16] Activation of JNK has been reported in various pathological conditions such as heart failure and ischaemia–reperfusion injury.

In a recent study, authors evaluated images from histologically stained tissue sections obtained from rabbit and human atria.[17] In this study, interstitial fibrosis was evaluated by Masson's trichrome stain. Fibrosis was not expected in our study due to interruption of blood supply for two hours, therefore immunohistochemical examination was not used in our study.

Our study indicated that increased TNF-α protein expression was associated with the tourniquet group; although, when all groups were compared with each other, no significant differences were found (Fig. 3). In this scope, our findings suggest that clamp application is favourable to the use of a tourniquet. Longer tourniquet time and higher inflation pressure were associated with higher complication risk.[18] Also, higher age and co-morbidities, such as trauma, peripheral vascular disease and hypertension, elevated the rate of complications.[19] Debates therefore continue about the safety limits associated with pressure and duration. However, most authors suggest that 1.5 to two hours with 200–250 mmHg inflation pressure is appropriate for healthy, normotensive patients.[8] When these safety limits are exceeded, complications may be encountered.[2]

Another parameter that affects the pressure is the cuff width. The cuff should be as wide as possible, and it should not encroach upon the surgical site.[2] Contrary to this general belief, a report suggests that muscle damage increases with wide cuffs.[20]

There are several reports regarding nerve injury related to the use of tourniquets.[21,22] Nerve tissue is more sensitive to mechanical pressure than muscle tissue, and two studies showed that injury was severe at the proximal and distal edges because of shear stress.[18,22] There was a strong correlation between mechanical pressure duration and nerve injury. Even below 30 minutes of inflation time, paralysis has been reported. Also, after each 30-minute increase in duration, there was a three-fold increase in neurological complications.[22]

Muscle tissue is more sensitive to prolonged ischaemia than nerve tissue. Moreover, the injury is severe beneath the cuff.[8] Animal studies have shown that tourniquets are related to decreased muscle force beneath and distal to the cuff and are directly proportional to cuff pressure.[23]

Contrary to nerve and muscle complications, vascular complications due to tourniquet use are rare. However, some reports suggest the opposite. For example, Rush *et al*. found that direct pressure can cause fracture of plaque formation or thrombosis in atherosclerotic vessels.[24] DeLaurentis *et al*. suggested not to use tourniquets if there is a femoropopliteal aneurysm, femoral–popliteal bypass or calcification. They also concluded that ischaemic pressure necrosis is an additional mechanism of injury.[25] Another report recommends avoiding tourniquet use with poor distal pulses, capillary return or calcified vessels near the application field.[26] However, the reasons for this suggestion (whether because of tourniquet-caused fractures or distortion-traction during surgery) are unclear.[27] Although various types of skin-protection paddings have been produced, skin injury can be encountered at rates of 0.04–0.1%.[28]

Tourniquet application therefore has several disadvantages. Nerve and muscle injury are common complications and can occur, even with short inflation times. Complication rates increase when the applied pressure is not adjusted to systemic blood pressure, extremity diameter and cuff width. Tourniquets also require regular calibration and incorrect calibrations can cause serious complications. Vessel complications may be less rare, but additional nerve, muscle and skin complications should be noted.

No international quantitative unit can repeatedly be used in experimental studies to measure clamp pressure.[29] In an experimental study, to standardise the clamp pressure between the subjects, the authors noted the lowest notch number at which the clamp did not slip on the vessel but provided transient occlusion.[9] In the same manner, to standardise the pressure, we used the same micro-clamp, which is the smallest available, to occlude the vessel for all subjects.

There are also experimental studies examining vessel damage due to clamp application.[9] In an experimental study, four DeBakey vascular clamps were applied to eight carotid arteries of four adult sheep for durations of 15, 30, 45 and 60 minutes, respectively. A significant and ongoing increase in endothelial damage was seen at 15 minutes; the damage was maximal after 30 minutes. The authors concluded that there are four variables to determine the force needed to occlude a vessel: vessel diameter, blood pressure, vessel elasticity and blade contact area. The severity of injury varies according to duration, pressure, intraluminal flow pattern, plaques and vessel elasticity.[9]

There are some limitations associated with our study. First, this study did not include a group of participants that were allowed to live after the study (to investigate the late histological changes for both the tourniquet and clamp application). This group of animals could have provided more information about the amount of repair possible over time and would also have shed light on clinical adaptations. However, it is unclear how long subjects should be kept alive to assess long-term effects. Another limitation of the study was that we were unable to compare or equalise the pressures of the tourniquet and the clamp.

Conclusion

This study found no difference between the tourniquet and clamp methods regarding vessel injury. The tourniquet is not ideal for the proximal field of extremities. In addition, it might cause vessel complications in the presence of underlying vascular disease. Also, skin, muscle and nerve complications could be encountered. Complication rates increased when the applied pressure was not adjusted to systemic blood pressure, extremity diameter and cuff width. When using a clamp in clinical practice, iatrogenic vessel injuries may be encountered during vascular dissection and may require peri-operative vascular surgeon consultation. However, clamp-related complications can be avoided with careful dissection and the minimum pressure required for occlusion. The arterial clamp method can be safe and useful, without tourniquet-related complications, for proximal extremities, where there is not enough space for a tourniquet.

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