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Original Article

Histological changes of vascular clipping in Wistar rats

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ABSTRACT

Background: During aneurysm microsurgery, the aneurysmal sac is excluded from circulation by placing one or more clips at the base of the aneurysm. In some cases of complex aneurysms or subarachnoid hemorrhage history, transient clipping before definitive clipping is necessary. The closing force of the transient clip is less than the permanent clip; however, it is sufficient to stop circulation to the aneurysmal sac. The aim of the following work is to analyze and describe histological changes caused by transient and permanent clipping of the abdominal aorta in Wistar-type rats, to study the correlation between the closing force of the clip and the time, it remains on the vascular tissue structures.

Methods: Six groups were formed, with 10 rats each, whereby temporary clipping of the abdominal aorta was performed with subsequent sampling of the site where the vascular clip was placed. The groups were: control and temporary clipping with: 2, 5, 10, and 15 and permanent clipping with 5 min.

Results: Resection samples of the 3 µm thick aorta were obtained through the routine histological technique and special histochemical techniques (Masson's Trichrome and orcein) from the six groups. Transmural changes were found from Group II–VI.

Conclusion: There is a vascular histological effect after both transient and permanent clipping. The sum of time and strength of the clip induce vascular changes visible at 5 min.

Keywords: Endothelial lesion, Histological changes, Vascular clipping, Wistar rats, Neuropathology

INTRODUCTION

Intracranial aneurysms are a vascular abnormality that causes focal dilation in the arterial wall, secondary to a loss of the internal elastic lamina. They are usually found in areas of arterial bifurcation of the anterior circulation of the Willis polygon. They have an incidence of 1–2% of the world population, being responsible for 80–85% of cases of nontraumatic subarachnoid hemorrhage (SAH). The peak age when presented corresponds to 40–60 years of age, with a slight preference for the female gender, 3:1.^[2,3,8,12,14]

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There are two types of treatments for brain aneurysms: endovascular or microsurgical treatment. The aim of microsurgery is to exclude the aneurysmal sac from circulation by placing one or more clips at the base of the aneurysm. For some cases of complex aneurysms or SAH history, transient clipping before definitive clipping is necessary. The closing force of the transient clip is less than the permanent clip; however, it is sufficient to stop circulation to the aneurysmal sac.^[8,4,12,15]

The aim of the following work is to analyze and describe the histological changes caused by the transient and permanent clipping of the abdominal aorta in Wistar-type rats, to study the correlation between the closing force of the clip and the time, it remains on the structures of the vascular tissue.

MATERIALS AND METHODS

A descriptive, experimental, cross-sectional, and prospective study was carried out in a group of 60 WISTAR-type rats, weighing an average of 400 g.

In addition, a comprehensive bibliographic search was carried out on the PubMed platform using the following keywords: histological vascular change, temporal clipping, arterial wall, endothelial lesion, and vascular clip; obtaining only 12 articles for use from 1979–2022, as well as use of bibliography from the Science Direct platform for the management and anatomy of murine models in the laboratory.

The procedures were performed in the Laboratory of Microsurgical Neuroanatomy on the Second Anatomy Class in the School of Medicine at the University of Buenos Aires. The experimental work was approved by the Ethics Committee for the School of Pharmacy and Biochemistry at the Institute of Medical Sciences (Cudap number 35788/19, Resolution No. 2379). Furthermore, followed were the care standards described by the “International Guiding Principles for Biomedical Research Involving Animals” written by the Council for International Organizations of Medical Sciences (CIOMS) and the International Council for Laboratory Animal Science, Geneva 2012, was also met.

The work protocol, developed by our team, consisted of integrating six groups, with 10 rats each, on which temporary clipping of the abdominal aorta was performed with subsequent sampling of the site where the vascular clip was placed, which were arranged as follows:

- Group I: Control group
- Group II: Temporary clipping for 2 min
- Group III: Temporary clipping for 5 min
- Group IV: Temporary clipping for 10 min
- Group V: Temporary clipping for 15 min
- Group VI: Permanent clipping for 5 min

The surgical instruments used for this study were a NEWTON® brand microscope, MEC XXI model, 2000 series

with 200 mm lens, macro and microsurgical instruments, such as scalpel handle and blade No. 11, microsurgical scissors, dissection scissors, straight tip dissection tweezers, needle holder, Farabeuf separators, five straight temporary clips of 0.69 N, 2 of 6.5 mm, 1 of 8 mm, 1 of 9.5 mm, and 1 of 12.5 mm, three permanent clips, two straight ones of 11.5 mm and 13.5 mm with pressure of 1.67 N and a curved one of 11 mm with 1.47 N, clip applicator tweezers, latex template, sterile gauze, 3-0 vicryl suture, buffered polypropylene, and formalin test tubes. The surgical procedure and anatomical description were recorded with a Nikon D5500 camera with a 62 mm lens and TRiOPO speedlight TR-15EX flash [Figure 1].

RESULTS

Anesthesia and positioning

The specimens were anesthetized with the following protocol: 80 mcg/kg of ketamine and 10 mg/kg of xylacin, intraperitoneally in the same syringe.

The rats were positioned in dorsal decubitus, with shaving of the abdominal region, asepsis and antisepsis with chlorhexidine, and marking of the incision. Once the procedure was completed, the specimens were euthanized with intraperitoneal barbiturate injection as required by international guidelines in the handling of laboratory animals.

Dissection of the abdominal region

A linear incision was made from the xiphoid process as the upper limit to the pubic symphysis [Figure 2a], taking the alba line as a reference point, until exposing the abdominal fascia [Figure 2b]. Dieresis of the muscle and peritoneum in a single plane, on the midline, achieving complete exposure of the abdominal cavity. Once the intestinal loops were identified [Figure 2c], they were moved to the right to be able to advance toward the deep planes until the retroperitoneal fat was recognized. A careful dissection was performed exposing the abdominal aortic artery to the left and the inferior vena cava to the right [Figure 2d].

Procedure and methodology of temporary and permanent clippings.

Dissection of the abdominal aorta was performed with the aforementioned technique and following the anatomical repairs mentioned. With adequate exposure thereof, a rectangular latex pad was placed below the blood vessel. In Group II, temporary clipping was initiated for 2 min with subsequent sampling of the vascular site where the clip was placed. The same procedure was repeated in subsequent specimens until the five working groups were completed, for 2, 5, 10, and 15 min of temporary clipping and 5 min of

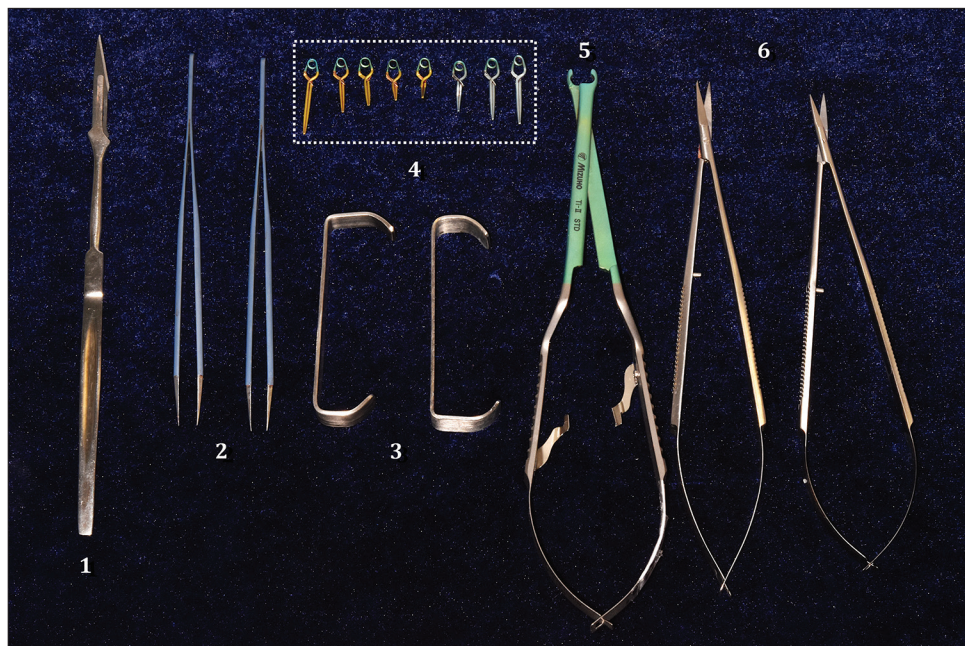


Figure 1: Microsurgical instruments. (1) Scalpel Handle with Blade No. 11. (2) Watchmaker tweezers. (3) Farabeuf separator. (4) Sugita Type II permanent and temporary clips in dashed rectangle line: five straight temporary clips in gold color of 0.69 N, 2 of 6.5 mm, 1 of 8 mm, 1 of 9.5 mm, and 1 of 12.5 mm, three permanent clips in silver color, two straight ones of 11.5 mm and 13.5mm with pressure of 1.67 N, and one curved one of 11 mm with 1.47 N. (5) Clip applicator clamp. (6) Microsurgical scissors.

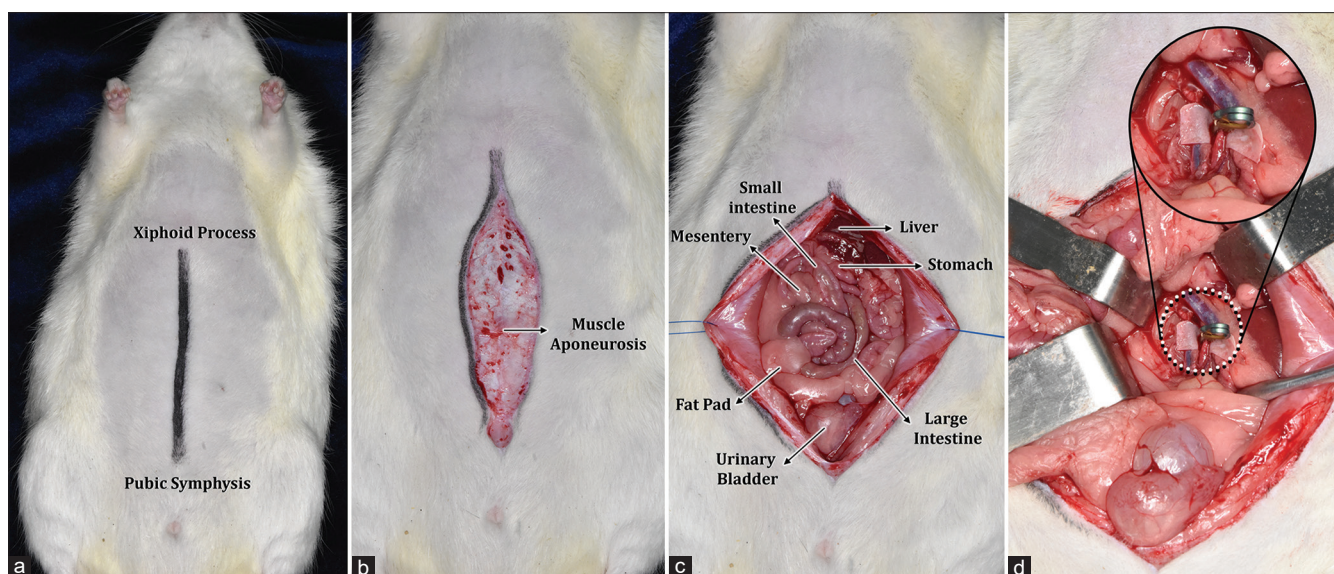


Figure 2: Abdominal dissection. (a) Linear incision marking. (b) Exposure of abdominal fascia. (c) Exposure of the abdominal cavity and contents. (d) Location of abdominal aortic artery to the left of the specimen with transient clip placed and inferior vena cava dissected to the right.

permanent clipping in Group VI. Finally, we worked with the control group, taking samples of the unharmed blood vessel. The sample was obtained by resection of the abdominal aortic artery in rodents after application of permanent and transient metal clips for a period of 2 min, 5 min, 10 min, and

15 min in addition to the control. During the macroscopic examination of the fresh samples, cuts were made at the level of the transition between the clips, separated into six (n : 6) groups (transient metal clips of 2 min, 5 min, 10 min, 15 min, and the permanent metal clips and control) and subsequently

fixed in buffered formaldehyde. The samples were processed in an automated manner and carefully included in paraffin for their correct orientation.

Histological cuts were made with a rotary microtome at 3 μm of thickness with routine histological technique (H&E) and special histochemical techniques (Masson's Trichrome and orcein). During the microscopic examination, the traumatic alterations produced in the arterial wall were evaluated and documented through microphotographs.

In Group I of the control sample, the integrity of the tissues is evidenced, with tunica adventitia, tunica media, and tunica intima without thickening or evidence of fibrinoid deposits. [Figure 3] In the transient clipping group of 2 min, 5 min, and 10 min, transmural parietal rupture was observed without folding and gradual increase of fibrinoid deposits in the tunica adventitia. [Figure 4] The histological sections examined by routine techniques (H&E) and special histochemical techniques (Masson's Trichrome and Orcein) showed, in the transverse section of the aorta in the permanent clipping group and 15-min transient clipping group, transmural parietal rupture with fibrinoid deposits in the tunica adventitia and internal folding of the arterial wall at the level of the rupture zone [Figures 5 and 6].

In none of the examined groups were endothelial lesions or subendothelial thickening identified exclusively, nor fragmentation of the tunica media or elastic lamina exclusively. With regards to the observed findings, transmural parietal rupture with associated parietal folding and fibrin deposits was identified in the groups that had permanent

and transient clippings for 15 min, so it is concluded that the parietal damage is intimately related to the strength of the clip and the length of time, it remains in place. However, this study did not analyze parietal alterations of long-term transient or permanent clippings.

DISCUSSION

Since Cushing's first description in 1911, vascular clips have become a necessary part of modern neurosurgery. Mc Kenzie in 1927 and Duane in 1950 made major changes to their material and shape. In 1953, Drew designed a special clip applicator that is still widely used today. In 1953, Norlen and Olivecrona developed a wire clip that could be reopened by applying support to the rear axis of the clip. In 1969, Heifetz designed a clip with an internal wire spring which reduced the fatigue of the clip. In 1971, Mayfield and Kees improved the design of Schwartz's cross-leaf clip, and the resulting Mayfield clip with silicone-covered tips was the most widely used spring clip for some time.^[13]

In 1928, Pool reported that Jefferson performed the first provisional ligation of the proximal artery, while Gibbs in 1957 during neck dissection of an aneurysm performed a temporary ligation using a modified Cairns clip. In 1969, Suzuki *et al.* measured for the first time, in 215 patients with aneurysms, the maximum safe time limit for cerebral ischemia when performing a temporary clipping with moderate hypothermia. In 1983, Bae *et al.* reported that temporary clipping of the middle cerebral artery in 16 patients could withstand up to 20 min to minimize complications.^[1]

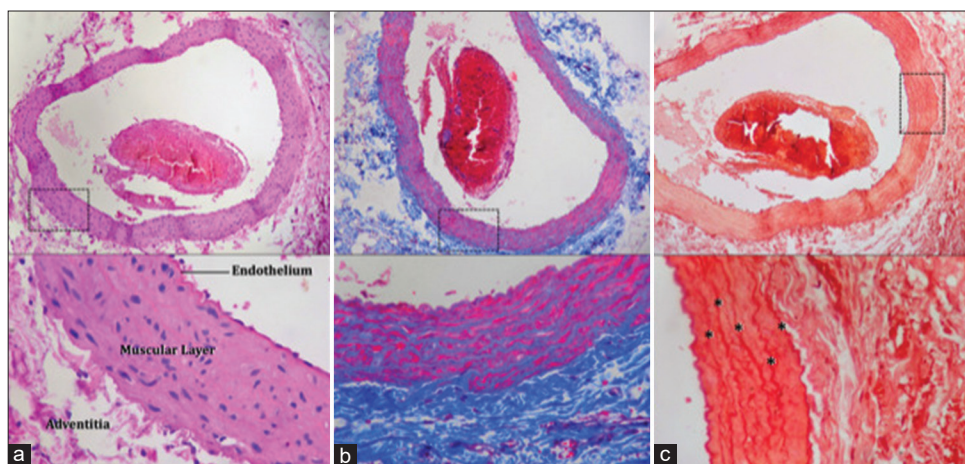


Figure 3: Control Group I. (a) $\times 10$ magnification with H&E staining: adventitia, middle tunic, and intima tunic are observed without thickening of the layers, vessel wall with blood content and connective tissue. (b) $\times 10$ magnification with Masson's Trichrome staining: the vessel wall is observed, tunica adventitia, tunica media, and tunica intima without thickening, with evidence in blue of normal elastic fibers without thickening them. (c) $\times 10$ magnification with Orcein stain: fragmentation of the blood vessel and marking of the elastic lamina with asterisks is observed. Dotted squares show an approach to the tissue shown in the figures.

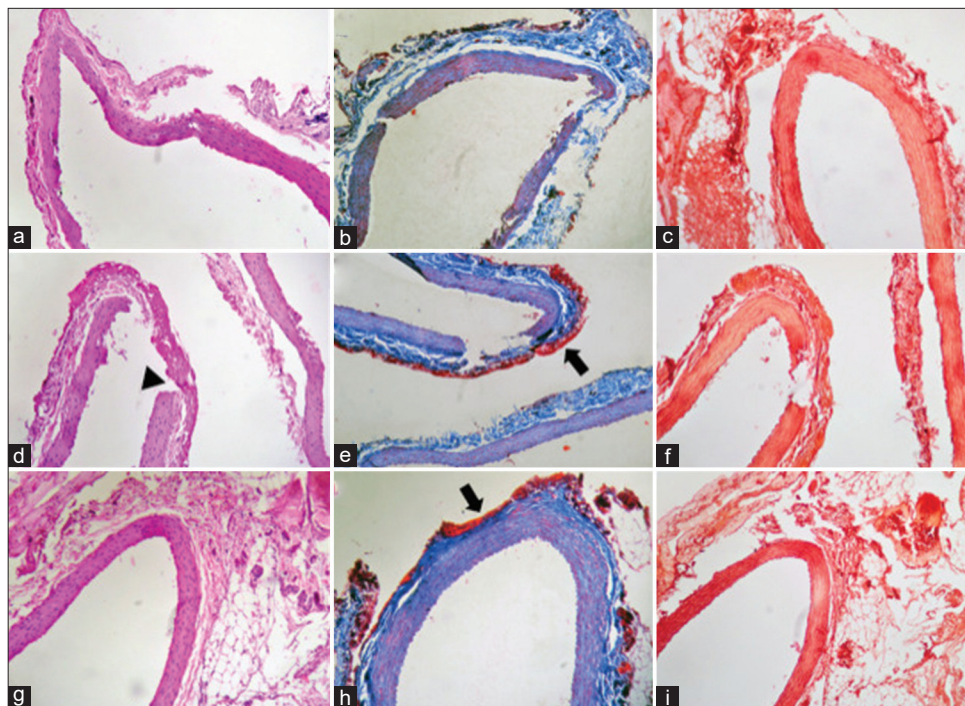


Figure 4: 2, 5, and 10 min Clipping. (a-c) 2-min clipping (a) 10x magnification of H&E staining: tunica adventitia, tunica media, and tunica intima are observed without thickening of the layers, connective tissue with fragmentation of the wall without fibrin deposit in the tunica adventitia. (b) $\times 10$ magnification of Masson's Trichrome staining: the vessel wall is observed, tunica adventitia, tunica media, and tunica intima without thickening. (c) $\times 10$ magnification of Orcein staining: no changes or rupture of the elastic lamina are observed. (d-f) Clip of 5 min. (d) $\times 10$ magnification of H&E staining: transmural rupture secondary to the mechanical trauma of the clip is observed, which presents a different morphology marked with an arrowhead image. Scarce fibrinoid deposit at the site of rupture, with no data of inflammatory infiltrate or microhemorrhage. (e) $\times 10$ magnification of Masson's Trichrome staining: fibrin deposit marked with arrow. (f) $\times 10$ magnification of Orcein staining: deposit observed with transmural rupture secondary to clip trauma. Separation of the muscle lamina and the fibrin layer unrelated to the time of clipping. (g-i) 10-min clipping (g) $\times 10$ magnification of H&E staining: blood vessel without significant changes. (h) $\times 10$ magnification of Masson's Trichrome staining: no evidence of transmural rupture but with presence of fibrin deposit (in red). The arrow signs fibrin deposits. (i) $\times 10$ magnification of Orcein staining: elastic lamina without thickening or changes.

Dujovny *et al.* demonstrated four levels of damage in the endothelial wall after temporary clipping: 1. crushing, 2. disruption, 3. disruption with clot formation, and 4. fracture with exposure of the tunica media, directly linked to the closing force of the vascular clip,^[6] which coincided with our histopathological findings, reaffirming that the severity of the vascular damage was directly proportional to the intensity of force applied by the clip and the length thereof.

On the other hand, Gertz *et al.* in 1976, in a study of 25 white rabbits where they worked with temporary clipping for 5, 15, and 30 min as well as 2 h, found that the carotid arteries that were occluded did not macroscopically exhibit any changes, although on examination under scanning and transmission electron microscopy on the compression surface, variation in the severity of lesions was observed, from distortions of the endothelial surface of the vessel,

up to cell fragmentation that compromised endothelial continuity with exposure of the subendothelial connective tissue. These alterations were detected in arterial segments that were clipped for at least 5 min; however, they did not change in frequency or increase in severity with long clippings of 30 min or 2 h,^[8] as opposed to our results that demonstrated a significant progression of transmural parietal rupture at longer clipping time.

As far as the effect of the clipping time, Dodson *et al.* worked on animals where the artery was occluded for half hour and 1 h, showing that the changes were more prominent in proximal clipping areas rather than in distal ones, consistent with myonecrosis. It was concluded that the initial alterations were induced by the production of chemicals by nerve damage and stasis of vascular elements and/or direct trauma to the wall.^[5]

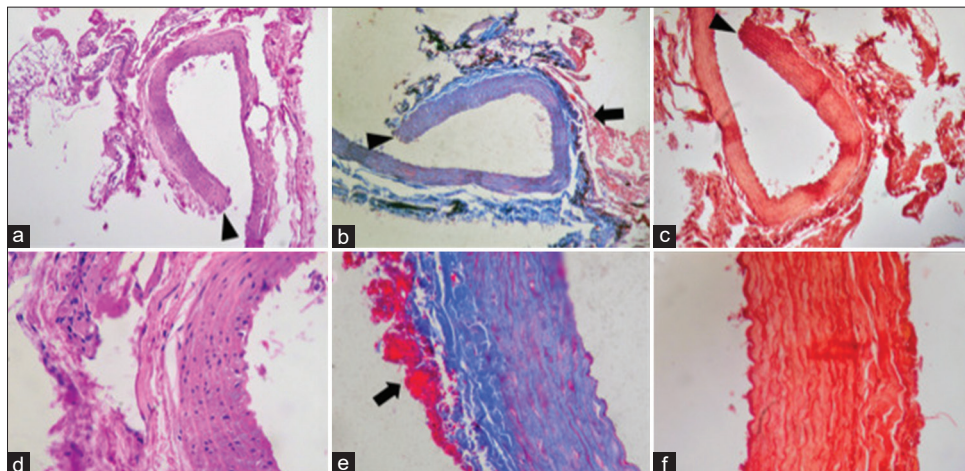


Figure 5: 15-min CLIPPING. (a) $\times 10$ magnification of H&E staining: transmurular rupture of the vascular wall is observed with increased uptake of staining by evidence of fibrinoid deposit in the adventitia layer. Arrowhead points out in the transmurular rupture. (b) $\times 10$ magnification of Masson's Trichrome staining: parietal rupture with fibrin deposit. Arrowhead transmurular rupture and arrow fibrin deposits. (c) $\times 10$ magnification of Orcein staining: transmurular rupture is observed. Arrowhead point out parietal rupture. (d) $\times 40$ magnification of H&E staining: marked fibrin deposit in the adventitia layer. (e) $\times 40$ magnification of H&E staining: integrity of the blood vessel with fibrin deposit (in red). Arrow point out fibrin deposits. (f) $\times 40$ magnification of Orcein staining: elastic lamina are stained, but without thickening or significant changes in them.

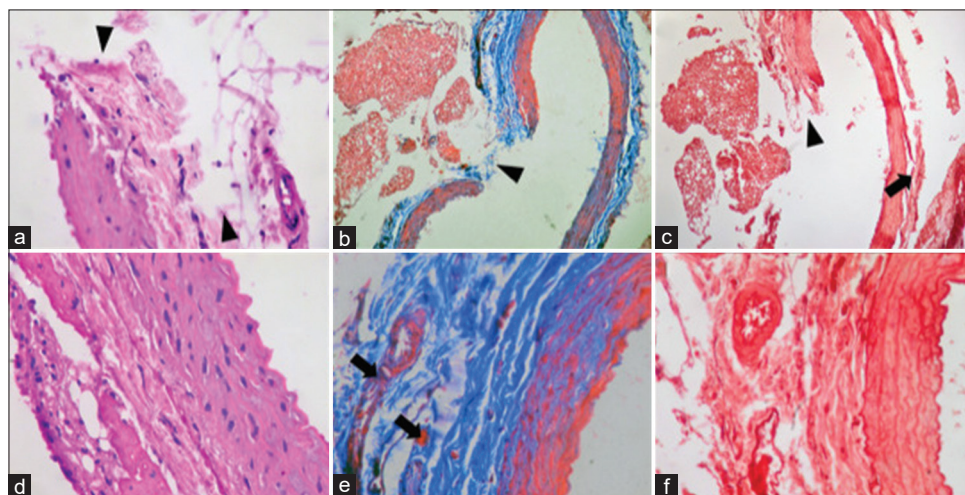


Figure 6: Permanent clipping. (a) $\times 40$ magnification of H&E staining: site of rupture of the entire vascular wall, observing continuity solution in the three layers with changes in morphology compared to the ruptures of 2, 5, and 10 min (arrowhead). (b) $\times 10$ magnification of Masson's Trichrome staining: continuity solution of the vascular wall is evidenced, with abundant fibrinoid deposit. Arrowhead point out with solution of continuity and fibrin deposit. (c) $\times 10$ magnification of Orcein staining: continuity solution in the entire vessel wall (arrowhead) and rupture of the muscle lamina in the lateral region of the vessel (black arrow). (d) $\times 40$ magnification of H&E staining: fibrin deposit is observed in the adventitia without thickening of the other layers. (e) $\times 40$ magnification of Masson's Trichrome staining: disruption of the elastic lamina with fibrin deposit in the adventitia layer (black arrow). (f) Increase $\times 40$ Orcein staining: elastic lamina are evidenced, without changes or thickening, only the fibrin deposit of adventitia.

Koh and Kwon in 2007, in an experimental study of 25 rats, which underwent continuous and intermittent temporal clipping, observed significant changes in the vessel wall

with continuous clipping above 5 min, reporting moderate endothelial cell damage, as well as disruption of adventitia at 10 min of continuous clipping and loss of endothelial

cells and segmental damage of muscle fibers at 15 and 20 min.^[10]

In 1982, Ebina *et al.* performed 17 postsurgical autopsies of permanent clippings for a period of time of 6 days–11 months, where it is observed that the granulation tissue around the vascular clip occurred beyond 1 month, the aneurysm lumen was also observed in cases beyond 13 days of clipping, showing formation of mural thrombus and fibrin deposit, as well as thickening of the tunica intima. After 1 month, the thickening of the tunica intima was fuzzy and it had organized thrombus, while at 3 months or more, the lumen of the aneurysm was almost completely closed due to thickening of the tunica intima, which could not be evaluated over time in our work.^[7]

Zhou *et al.* in 1993 have already mentioned that with regards to temporary arterial occlusion and the risk of damage to the blood vessel wall, the relationship between damage to the arterial wall, the contact area of the clip, the closing pressure of the clip, the diameter of the blood vessel, the elasticity of the blood vessel, the blood pressure and the time of occlusion was well known.^[11]

Hoff and Gottlob stated that even transient clipping caused an abnormal overlap of endothelial cells and the formation of pseudopods of smooth muscle cells that were projected into the light, which were reversible changes. The irreversible changes were necrosis and thickening of the intima cells, retention of fibrin, cholesterol, etc. in the subendothelial space and fragmentation of the elastic lamina.^[9]

Limitations

One of the limitations of this work is the vascular anatomical variability of our experimental model in comparison to that of the human species, since a difference was observed in the muscle layer of Wistar rats, which has multiple concentric elastic lamina, while the intracerebral blood vessels of humans have an internal and external muscle layer.

Another pending aspect to be analyzed is the effect of transient and permanent long-term clipping and observation is required of the postsurgical effect of clipping over time with monthly monitoring, to evaluate if there is remodeling of the vessel or exacerbation of histological damage.

We believe that this type of experimental work motivates the future studies, which should examine in detail if the histological damage is directly related to the trauma of the clipping's time and closing force or if it is the result of sustained ischemia of the *vasa vasorum*.

CONCLUSION

Based on the series of papers published to date, whose results coincided with our findings, the histological vascular effect

after both transient and permanent clipping is indisputable, which makes it essential to understand the effect of the clipping force as well as its application time on the vascular endothelium.

It is concluded that the sum of time and force in the clipping application induces objectifiable vascular changes after 5 min, which is why it would be recommendable to avoid exceeding this length of transient clipping time and reduce the possibility of rearranging the permanent clip in the same site where it has been placed.

Declaration of patient consent

The authors certify that they have obtained all appropriate consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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