Radiographic and Histological Evaluation in Canine Femur after Implantation of 304 Stainless-steel-based Plate

Agus Purnomo1, Hartiningsih1, Setyo Budhi1, Dhirgo Adji1, Devita Anggraeni1, Dito Anggoro1, Sitarina Widyarini1, Shekhar Chhetri2, Muhammad Thohawi Elziyad Purnama4,5,6

1Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, INDONESIA
2Pharmacognosy Journal, Vol 14, Issue 4, July-Aug, 2022
3Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, INDONESIA
4Department of Animal Science, College of Natural Resources, Royal University of Bhutan, Lobesa, Punakha, BHUTAN.
5Division of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, INDONESIA
6School of Health and Life Sciences, Universitas Airlangga, Surabaya, INDONESIA

Correspondence
Muhammad Thohawi Elziyad Purnama
Division of Veterinary Anatomy, Faculty of Veterinary Medicine, School of Health and Life Sciences, Universitas Airlangga, Surabaya, INDONESIA.
E-mail: thohawi@fkh.unair.ac.id

ABSTRACT
Fractures are known to be high-risk traumatic cases in domestic animals. Surgery was performed to reposition and immobilize bone using a plate as a biomaterial component. This study aimed to evaluate the radiographic and histological findings in canine femur after implantation of a 304 stainless-steel-based plate. A total of six male dogs aged 3-4 months were used in this study. Dogs were acclimatized for a week and then randomly assigned to 2 groups and 3 replication, respectively. The left femoral diaphysis was cracked and fixed by (A) commercial plate; (B) 304 stainless-steel plate. Radiographic observations were performed at 24 h and 28 d postoperatively during the recovery period. Histological figures were evaluated at 28 d postoperative descriptively. As a result, physical examination of muscle tissue in both groups did not reveal discoloration, swelling, and fluid accumulation around the plate. The radiographic figures presented a slight callus production around the crack and a decrease in the gap between cracks after 28 d. The histological figures observed the proliferation of osteoblasts, osteocytes, connective tissues, and bone trabeculae. It can be concluded that no significant difference between 304 stainless-steel plate compared to commercial plate. A 304 stainless-steel-based plate is recommended for bone immobilization in canine femoral fractures.

Key words: Domesticated animal, Femur, Fracture, Radiograph, 304 stainless-steel.

INTRODUCTION
Femoral fractures are common in dogs with a prevalence of 31.9% in Cairo, Egypt,1 17.4% in India,1 50% in Brazil,2 and 57.7% in Ibadan, Nigeria.3 Fracture is a break in the continuity of bone and cartilage tissue which is generally caused by trauma. Fractures are characterized by substantive soft tissue damage, separation of the periosteum from the bone, extensive bleeding, lacerations and muscular damage.4 Severe bone damage due to trauma, where many fracture fragments cannot be maintained so that it can inhibit bone healing. In addition, delayed healing or non-union complications after surgery are also causes of bone damage. In general, simple fractures are easily treated by fixing the fracture fragments using intramedullary pins or external fixators.5

The application of implant materials as endoprosthetics in animals requires good biocompatibility, strength, and resistance to corrosion, especially body fluids. Materials commonly used as implant materials are stainless steel, Cobalt-based metal alloys and titanium.6 Titanium alloys have good biocompatibility and corrosion resistance, but are very expensive. Stainless steel has a lower level of biocompatibility than titanium alloys and cobalt–chromium–molybdenum alloy (CoCrMo). However, it has good mechanical properties and is cheaper than titanium and CoCrMo alloys. The basic requirements of metal-based biomaterials are low corrosive properties and must have biocompatibility.6

This is important because biomaterials are implanted in the body and are in direct connection with living cells. Metals used as biomaterials must not release ions that are toxic or carcinogenic to living cells. Corrosion reactions on implant materials can cause an inflammatory reaction around the tissue so that if used in the long term it will be very dangerous for the body. A bone plate is one of the components of implants that are made to replace bone structure and function and support fractures.7 In the context of this study, implant materials can be applied internally or externally. The aim of this study was to evaluate the radiographic and histological findings in canine femur after implantation of a 304 stainless-steel-based plate.

MATERIALS AND METHODS
This study was approved by the Ethical committee: Animal care and laboratory use, Faculty of Veterinary Medicine, Universitas Gadjah Mada with Certificate No.0098/EC-FKH/Int./2019. Ethical approval in this study is required to prevent stress and animal abuse.

A total of 6 local male dogs aged 3-4 months, weighing 4-5 kg, were reared in individual cages, Department of Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Dogs were anesthetized for a week and fed commercial feed (Rotto®, Thailand), drinking ad libitum and 25 mg/kg of anthelmintic Pyrantel pamoate was administered. After acclimatization, dogs were assigned into 2 groups and 3 replications i.e. (A) commercial plate and (B) 304 stainless-steel plate, respectively.

Prior to surgery, dogs fasted for 12 h, then 0.04 mg/Kg BW premedication was injected subcutaneously with atropine sulfate. After 15 minutes, the dog was anesthetized using a combination of 10 mg/kg BW of ketamine and 2 mg/kg BW of xylazine HCL.

Cite this article: Purnomo A, Hartiningsih, Budhi S, Adji D, Anggraeni D, Anggoro D, et al. Radiographic and Histological Evaluation in Canine Femur after Implantation of 304 Stainless-steel-based Plate. Pharmacogn J. 2022;14(4): 388-392.
intramuscularly. The dog was positioned in right lateral recumbency, then the hair in the left femur area was shaved and cleaned with 70% alcohol and povidone-iodine solution. The sterile drape was covered over the whole body of the dog except the head area. The skin incision was performed along the cranialateral bone in the line from the greater trochanter to the patella, as well as the subcutaneous tissue. The skin and subcutaneous tissue were retracted, the fascia lata was incised along the cranial margin of the biceps femoris muscle. The biceps femoris muscle was retracted caudally and the vastus lateralis muscle was retracted cranially so that the surface of the femur bone will be seen. In both groups, the left femoral diaphysis was cracked and fixed with a plate as assigned. Thereafter, the muscle was sutured using Vicryl and the skin using silk. Radiographic observations were performed at 24 h and 28 d postoperatively. A bone biopsy was performed on day 28 and fixed in 10% buffered formalin for histological evaluation using hematoxylin and eosin (HE) staining. Histological analysis was performed using a binocular microscope (Eclipse E200 LED, Nikon, Japan). The following variables were observed i.e. newly formed trabecular bone, connective tissue (including fibrous tissue, blood vessels, and adipose tissue), total tissue volume, osteogenic cells (including osteoblast and osteoclast). All data were expressed as mean ± standard error and analyzed using T-test independent sample (p<0.05).

RESULTS
After 24 h, radiographs in both groups showed radiolucent findings with a 1 mm gap representing bone fragments in the left femoral diaphysis and no callus production (Figure 1). Meanwhile, after 28 d, radiolucent findings were observed without gaps and callus production was initiated. The callus production was not excessive around the radiopaque visible fragments indicating a mineralization process (Figure 2). On the other hand, physical examination of the plates in both groups showed no change in muscle coloration or excessive fluid production in post-implantation bone tissue (Figure 3).

In this case, it showed that there was no excessive inflammation reaction and no rejection effect in the implantation of 304 stainless-steel plates. In the present study, the histologic findings at 28 d observed proliferation of fibrous connective tissue, trabecular bone formation, new vascularization, osteoblasts at the bone margins of the trabeculae, and osteocytes in the center of the bone matrix (Figure 4). The muscles around the fracture area showed no inflammation, myopathy, or atrophy. In addition, the new formation of trabecular bone, connective tissue, and osteogenic cells appeared similar in the two treatment groups (Table 1).
vascularization from the host to the mid-fracture area and terminated proliferation at the border of trabecular bone. The occurrence of fibrous tissue proliferation, similar formations, i.e. histological evaluation 28 d postoperatively, both groups showed cells (MSCs), osteoblast proliferation and macrophage chemotaxis. Cytokines and promote the healing stage. Hematomas production requires ischemic environmental conditions, and then decrease in 1-2 weeks. The inflammatory phase is initiated immediately after bone injury and surrounding soft tissue.

In the case of fractures, tissue disorders can occur in cells, blood vessels, bone matrix, muscles, and nerves. Bleeding occurs from the periosteum, endosteum, soft tissue around the fractured bone, and can also be caused by large blood vessels. The hematoma will develop at the fracture site for several hours on the first day. Hematoma consists of platelets and macrophages which are stimulated to release a series of cytokines and promote the healing stage.

The types of cytokines involved in the healing stage are platelet derived growth factor (PDGF), transforming growth factor beta group of protein (TGF-β), interleukin-1 (IL-1), interleukin-6 (IL-6), and prostaglandin E2 (PG-E2). Fibrin clot platelets degranulate releasing TGF-β and PDGF. Furthermore, TGF-β stimulates mesenchymal stem cells (MSCs), osteoblast proliferation and macrophage chemotaxis. Hematomas production requires ischemic environmental conditions, hypoxia, low pH and high concentrations of potassium and lactate.

The hematoma may produce a buffer before the growth of fibrous tissue and provide fibrin stability. The inflammatory reaction occurs at the fracture site by releasing lysosomal enzymes from the fracture border and soft tissue. Clinically, the end of this inflammatory phase is marked by decreased pain and swelling. This phase takes about 1-7 days. The inflammatory phase prolongs the fracture time until it produces a bony callus. Radiographically it can be observed that the end of the fracture looks less opaque and the edges rather vague. Healing progress was observed after 28 d postoperative, in which the bone fragments were not visible and were filled with callus. The callus production formed is characterized by a radiopaque appearance that is not excessive on X-rays. After that, the callus formed will be mineralized so that the radiograph looks more radiopaque.

Bone healing depends on an adequate vascular supply and supported by osteoblasts modulation around the blood vessels so that the formation of bone tissue is more organized on a stable and solid surface. The initial stage of bone union is callus formation, followed by vascularization from the host to the mid-fracture area and terminated by bone matrix resorption and new bone replacement. Based on histological evaluation 28 d postoperatively, both groups showed similar formations, i.e. the occurrence of fibrous tissue proliferation, trabecular bone formation, stimulation of angiogenesis, and osteoblast proliferation at the border of trabecular bone.

Subsequently, fibroblasts develop and infiltrate blood capillaries into the blood clot to form granulation tissue called procalus. Granulation tissue will develop dense fibrous tissue and turn into cartilage. This tissue is a temporary callus to hold the fractured bone. The temporary callus is then progressively replaced by spong bone derived from the osteoblasts proliferation.

Vascularization that occurs in the fracture area is an indication of the healing process. Bone healing from vascularization will be initiated immediately after reconstruction because the periosteal blood supply is complete after the vascular anastomosis. Inflammatory cells will aggregate in the fracture area, then monocytes that enter the fracture area will transform into macrophages which play an important role in bone healing. This will lead to granulation tissue formation, neovascularization, and migration of osteogenic cells.

Histological picture of the tissue around the implanted plate depicts the absence of inflammatory cells, myopathy or muscular atrophy. These findings indicate that the universal use of 304 stainless-steel-based bone plate does not have a negative effect on the healing process or the surrounding tissue. 304 stainless steel is an austenitic category that has a face-centered-cubic (fcc) structure so that it is superior to ferritic stainless steel in terms of corrosion resistance due to its higher crystallographic atomic density, yield strength ratio and very low tensile test.

Type 316L stainless-steel is the most commonly used material for implant materials. 316L stainless-steel is a low carbon type with chemical composition of ≤0.030% carbon, ≤1.0% silicon, ≤2.0% manganese, ≤0.045% phosphorus, ≤0.030% sulfur, 12.0-15.0% nickel, and 16.0-18.0% chromium. Another type of alloy metal is CoCr alloy consisting of 65% cobalt and 30% chromium with a small amount of carbon element which has a hard, rigid, strong texture and excellent corrosion resistance.

CONCLUSIONS

In conclusion, non-commercial plates of 304 stainless-steel-based have no impact on inflammatory reactions at the implant site. In addition, the proliferation of osteoblasts, osteocytes, and bone trabecular proved primary ossification in the canine femur during the healing period.

ACKNOWLEDGEMENTS

The author would like to thank the Faculty of Veterinary Medicine, Universitas Gadjah Mada for funding this study with grant number 1011/J01.1.22/HK4/2019.

DECLARATION OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Elzomor ST, Sheta EME, Farghali HA, Ashour AE. Prevalence of femoral fractures in dogs and cats. J Egypt Vet Med Assoc. 2014;72(2):269-278.
2. Acharya AS, Ravneet K, Kulwant L. Rabies epidemiology and control in India: A review. J Commun Dis. 2012;44(2):59-68.
3. Libardoni RD, Sarafini GMC, Oliveira CD, Schmites PI, Chaves RO, Feranti JPS, et al. Appendicular fractures of traumatic etiology in dogs: 955 cases (2004-2013). Ciência Rural. 2016;46(2):542-548.
4. Eyarefe OD, Oyetayo SN. Prevalence and pattern of small animal orthopaedic conditions at the Veterinary Teaching Hospital, University of Ibadan. Sokoto J Vet Sci. 2016;14(2):8-15.
5. Utvåg SE, Grundnes O, Rindal DB, Reikerås O. Influence of extensive muscle injury on fracture healing in rat tibia. J Orthop Trauma. 2003;17(6):430-435.
6. Canapp SO. External fracture fixation. Clin Tech Small Anim Pract. 2004;19(3):114-119.
7. Thomas P, Bandi WD, Maier S, Summer B, Przybilla B. Hypersensitivity to titanium osteosynthesis with impaired fracture healing, eczema, and T-cell hyperresponsiveness in vitro: case report and review of the literature. Contact Dermat. 2006;55(4):199-202.

8. Aherwar A, Singh AK, Patnaik A. Cobalt Based Alloy: A Better Choice Biomaterial for Hip Implants. Trends Biomater Artif Organs. 2016;30(1):133-137.

9. Maurus PB, Kaeding CC. Bioabsorbable implant material review. Oper Tech Sports Med. 2004;12(3):158-160.

10. Purnama MTE, Rahmainagtys IH, Pratama AR, Prastika Z, Kartikasari AM, Cahyo NPD. Tadpole serum activity (Rana catesbeiana) in caspase-3 as a marker of the role of apoptosis and total cytotoxic T lymphocytes in albino rats' epithelial cells induced by neoplasia. Vet World. 2019;12(1):63.

11. Schmidt-Bleek K, Kwee BJ, Mooney DJ, Duda GN. Boon and bane of inflammation in bone tissue regeneration and its link with angiogenesis. Tissue Eng Part B Rev. 2015;21(4):354-364.

12. Roga GE, Vashishth D. Effects of bone matrix proteins on fracture and fragility in osteoporosis. Curr Osteoporos Rep. 2012;10(2):141-150.

13. Kolar P, Gaber T, Perka C, Duda GN, Buttgerfeit F. Human early fracture hematoma is characterized by inflammation and hypoxia. Clin Orthop Relat Res. 2011;469(11):3118-3126.

14. McLear RR. Proinflammatory cytokines and osteoporosis. Curr Osteoporos Rep. 2009;7(4):134-139.

15. Fischer V, Kalbittz M, Müller-Graf F, Gebhard F, Ignatius A, Liedert A, et al. Influence of menopause on inflammatory cytokines during murine and human bone fracture healing. Int J Mol Sci. 2018;19(7):2070.

16. Engstrom M, Schott U, Nordstrom CH, Romner B, Reinstrup P. Increased lactate levels impair the coagulation system—a potential contributing factor to progressive hemorrhage after traumatic brain injury. J Neurosurg Anesthesiol. 2006;18(3):200-204.

17. Hamid IS, Ekowati J, Purnama MTE. Kaempferia galanga L. Inhibiting Effect on Vascular Endothelial Growth Factor (VEGF) and Cyclooxygenase-2 (COX-2) Expression on Endothelium of Chorioallantoic Membrane. Indian Vet J. 2019;66(9):80-82.

18. Mofid MM, Inoue N, Atabay A, Marti G, Chao EY, Manson PN, et al. Callus stimulation in distraction osteogenesis. Plast Reconstr Surg. 2002;109(5):1621-1629.

19. Cui FZ, Zhang Y, Wen HB, Zhu XD. Microstructural evolution in external callus of human long bone. Mater Sci Eng C. 2000;11(1):27-33.

20. Kim UK, Chung IK, Lee KH, Swift JQ, Seong WJ, Ko CC. Bone regeneration in mandibular distraction osteogenesis combined with compression stimulation. J Oral Maxillofacial Surg. 2006;64(10):1498-1505.

21. Strothbach CA, Rundle CH, Wergedal JE, Chet ST, Linkhart TA, Lau KH, et al. LMP-1 retrovirus gene therapy influences osteoblast differentiation and fracture repair: a preliminary study. Calcif Tissue Int. 2008;83(3):202-211.

22. Schindeler A, McDonald MM, Bokko P, Little DG. Bone remodeling during fracture repair: The cellular picture. In Seminars in cell & developmental biology. Academic Press. 2008;19(5):459-466.

23. Rutten S, Nolte PA, Korstjens CM, van Duin MA, Klein-Nulend J. Low-intensity pulsed ultrasound increases bone volume, osteoid thickness and mineral apposition rate in the area of fracture healing in patients with a delayed union of the osteotomized fibula. Bone. 2008;43(2):348-354.

24. Nakajima F, Nakajima A, Ogasawara A, Moriya H, Yamazaki M. Effects of a single percutaneous injection of basic fibroblast growth factor on the healing of a closed femoral shaft fracture in the rat. Calcif Tissue Int. 2007;81(2):132-138.

25. Keramaris NC, Calori GM, Nikolau VS, Schemitsch EH, Giannoudis PV. Fracture vascularity and bone healing: a systematic review of the role of VEGF. Injury. 2008;39(3):S45-S57.

26. Pountos I, Panteli M, Panagiotopoulos E, Jones E, Giannoudis PV. Can we enhance fracture vascularity: what is the evidence? Injury. 2014;45(4):S49-S57.

27. Pham HT, Iwamoto T. An evaluation of fracture properties of type-304 austenitic stainless steel at high deformation rate using the small punch test. Int J Mech Sci. 2018;144(1):249-261.

28. San Marchi C, Somerday BP, Tang X, Schiroky GH. Effects of alloy composition and strain hardening on tensile fracture of hydrogen-precharged type 316 stainless steels. Int J Hydrogen Energy. 2008;33(2):889-904.

29. Tokaji K, Kohyama K, Akita M. Fatigue behaviour and fracture mechanism of a 316 stainless steel hardened by carburizing. Int J Fatigue. 2004;26(5):543-551.
ABOUT AUTHORS

Agus Purnomo is a Lecturer and researcher at the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Research interest in surgery and radiology.

Hartiningsih is an Associate professor and researcher at the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Research interest in surgery and radiology.

Setyo Budhi is an Associate professor and researcher at the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Research interest in surgery and radiology.

Dhirgo Adji is an Associate professor and researcher at the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Research interest in surgery and radiology.

Devita Anggraeni is a Lecturer and researcher at the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Research interest in surgery and radiology.

Dito Anggoro is a Lecturer and researcher at the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Research interest in surgery and radiology.

Sitarina Widyarini is an Associate professor and researcher at the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Research interest in organ pathology, veterinary forensics and phytochemistry.

Shekhar Chhetri is a Lecturer and researcher at the Department of Animal Science, College of Natural Resources, Royal University of Bhutan. Research interest in domestic animal husbandry.

Muhammad Thohawi Elziyad Purnama is a Lecturer and researcher at the Division of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga. Research interests in anatomy, biomaterials and animal welfare.

Cite this article: Purnomo A, Hartiningsih, Budhi S, Adji D, Anggraeni D, Anggoro D, et al. Radiographic and Histological Evaluation in Canine Femur after Implantation of 304 Stainless-steel-based Plate. Pharmacogn J. 2022;14(4): 388-392.