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Research Article

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

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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.

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INTRODUCTION

Femoral fractures are common in dogs with a prevalence of 31.9% in Cairo, Egypt,[1] 17.4% in India,[2] 50% in Brazil,[3] and 57.7% in Ibadan, Nigeria.[4] 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.[5] 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.[6]

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.[7] 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.[8]

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.[9] 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 acclimatized 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

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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 craniolateral 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 lateral 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.[10]

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).

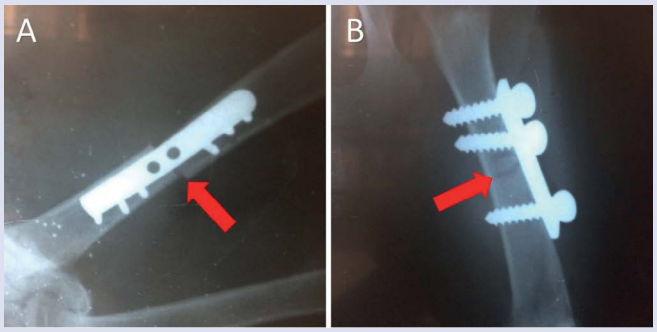

Figure 1: Radiograph of the left femur in (A) commercial plate group, (B) 304 stainless-steel plate group at 24 h postoperatively. The (→) arrows indicate radiolucency with a gap of 1 mm.

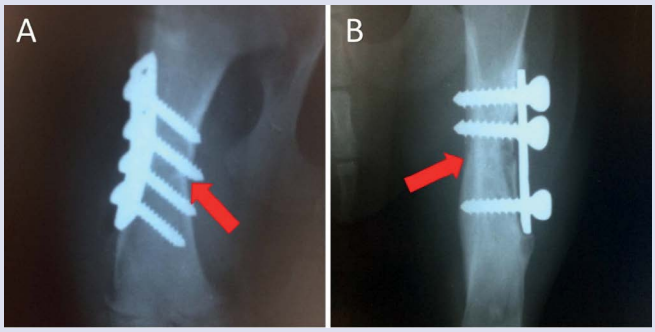

Figure 2: Radiograph of the left femur in (A) commercial plate group, (B) 304 stainless-steel plate group at 28 d postoperatively. The (→) arrows indicate radiolucency with callus formation and no gaps were found.

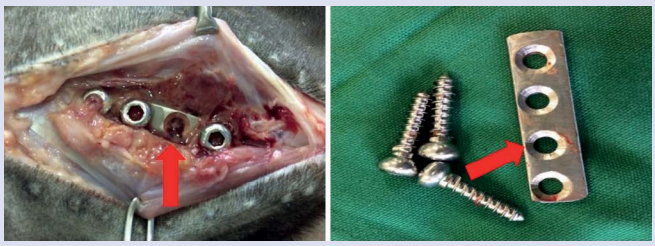

Figure 3: Physical examination of the plate macroscopically in the 304 stainless-steel plate group. The arrows indicate no change in muscle and plate discoloration after implantation.

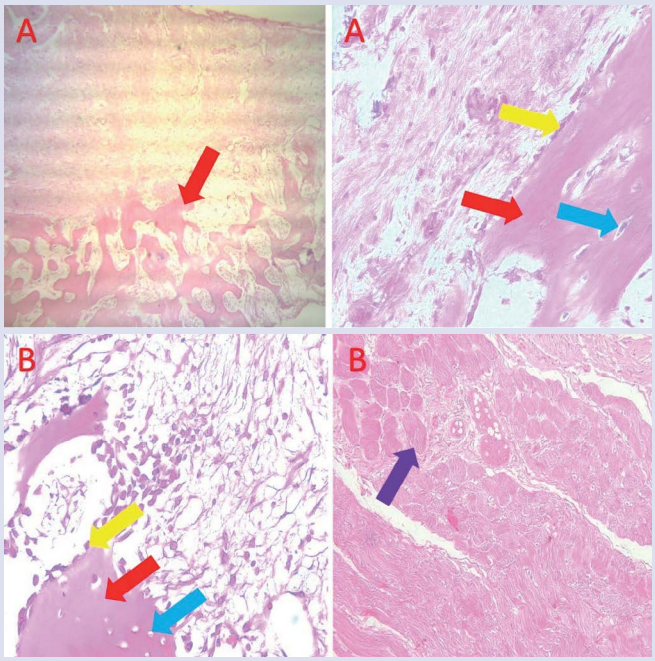

Figure 4: Histological finding of the left femur in (A) commercial plate group, (B) 304 stainless-steel plate group at 28 d postoperatively. The arrows indicate (→) proliferation of osteoblasts, (→) bone trabeculae, and (→) osteocytes. In muscle histology, (→) there was no inflammation, myopathy, and atrophy.

Table 1: Histological evaluation in bone biopsy after 28 d postoperative.

Variables	Commercial plate	304 stainless-steel plate	p-value
Newly formed trabecular bone	41.56 ± 0.49	42.10 ± 1.08	0.278
Connective tissue	31.65 ± 0.56	31.54 ± 0.32	0.406
Total tissue volume	81.07 ± 0.49	80.79 ± 0.66	0.476
Osteogenic cells	20.67 ± 0.88	21.37 ± 0.67	0.653

Values are expressed in mean ± standard error. T-test independent sample was carried out with significant differences for $p<0.05$.

DISCUSSION

Based on radiographic observations at 24 h postoperatively, it was revealed that both groups had not yet produced callus. Callus production depends on the end of the inflammatory phase. The inflammatory phase is a response that occurs when there is fracture, injury, and inflammation that can reach peak production at 48 h and then decrease in 1-2 weeks. The inflammatory phase is initiated immediately after bone injury and surrounding soft tissue.[11]

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.[12] 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.[13]

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).[14] Fibrin clot platelets degranulate releasing TGF-β and PDGF. Furthermore, TGF-β stimulates mesenchymal stem cells (MSCs), osteoblast proliferation and macrophage chemotaxis.[15] Hematomas production requires ischemic environmental conditions, hypoxia, low pH and high concentrations of potassium and lactate.[16]

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.[17] 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.[18] 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.[19] 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.[20]

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.[21] 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.[22] 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.[23]

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 spongy bone derived from the osteoblasts proliferation.[24]

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.[25] 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.[26]

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.[27]

Type 316L stainless-steel is the most commonly used material for implant materials. 316L stainless-steels 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.[28] 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.[29]

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 trabeculae proved primary ossification in the canine femur during the healing period.

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DECLARATION OF INTEREST

The authors declare no conflicts of interest.

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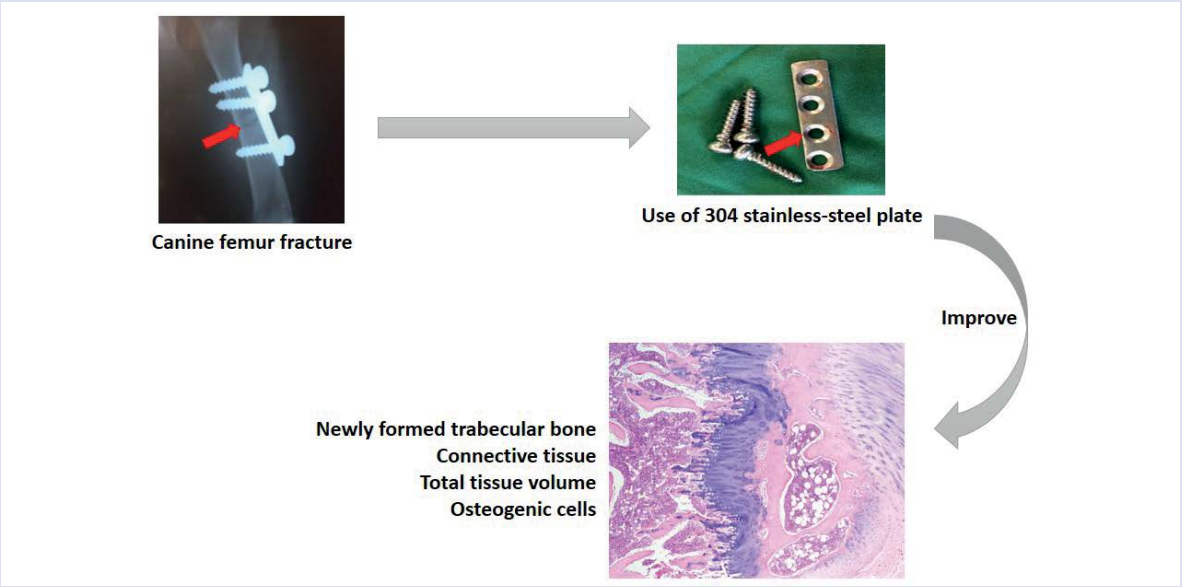
GRAPHICAL ABSTRACT



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