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Can bone marrow aspirate improve mandibular fracture repair in camels (*Camelus dromedarius*)? A preliminary study

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Conflict of Interest
The authors declare no conflicts of interest.

ABSTRACT

Background: Mandibular fractures are common in camels, leading to considerable economic losses. This study explored methods of improving mandibular fractures repair, adjuvant with interdental wire, or bone plate fixation. Autologous bone marrow (BM) injection enhances osteogenesis and rapid healing.
Objectives: To investigate the effect of autologous BM aspirate as an adjuvant treatment for repairing mandibular fractures in camels with interdental wire, or bone plate fixation.
Methods: Thirty dromedary camels aged 5–8 years and of both sexes were randomly divided into 4 treatment groups: group 1 (n = 10) treated with stainless steel wire fixation and BM injection at the fracture line, group 2 (n = 10) treated with plate fixation and BM injection at the fracture line, group 3 (n = 5) treated with stainless steel bone wire fixation and placebo saline injection at the fracture line, and group 4 (n = 5) treated with plate fixation and placebo injection at the fracture line. The mandibular fractures were followed weekly for 12 weeks postoperatively to assess improvement and healing based on clinical evaluation, radiographic union scale, and bone turnover markers (i.e., bone alkaline phosphatase, osteocalcin, pyridinoline, and deoxypyridinoline).
Results: Compared to other groups, elevated bone turnover markers in group 1 were demonstrated ($p < 0.05$) on the seventh postoperative day. Likewise, compared to other groups, both clinical findings and radiographic union scale significantly improved ($p < 0.05$) in group 1 on the 56th postoperative day.
Conclusions: BM aspirate has a promising beneficial osteogenic effect on mandibular fracture repair in camels, most notably when combined with interdental wire fixation.

Keywords: Bone marrow; mandibular fracture; camel; bone wire; bone plate

INTRODUCTION

Mandibular fracture is a common condition that occur in dromedary camels. Biting and fighting during rut season are considered as the leading causes of mandibular fracture in

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dromedary camels. Moreover, mandibular fractures also occur inadvertently (e.g., running into fixed objects, falling during a race, other accidents) [1,2]. The most common site of these fractures is the interdental space, just cranial or caudal to the first premolars [3]. However, bilateral compound and transverse fractures of the mandible are more common than unilateral ones in camels. Fractures of other parts of the mandible are considered atypical [4,5].

Mandibular fractures range from deep fractures involving a portion of the mandible and several incisor teeth to simple fractures with loss of a single tooth [6]. When fractured, ventral displacement of the fractured portion of the mandible occurs, with an open gingival wound on the floor of the mouth. Caudal or dorsal compression occasionally creates fracture fragments due to the mechanism of injury. Incisor teeth fracture is rare and is only encountered in bilateral fractures [7,8].

Different repair techniques have been reported for mandibular fractures in camels, including interdental wiring [3], reinforced brass interdental wiring [9], unilateral/ bilateral bone plating [4,10], transfixion using bone pins along with plaster of paris bandaging [5], splinting with a wooden plate along with plaster of paris bandaging [11], and surgical excision of an irreparably fractured mandibular segment [12]. However, these study results are controversial because of the prolonged time required for fracture repair, resulting infections, implant failure, and jaw deformity. These issues form the rationale of this study, which seeks a method by which the repair can be timely and successful.

The use of osteogenic progenitor cells has been encouraged when treating numerous orthopedic afflictions in animals. Bone marrow (BM) has become a reliable source of progenitor cells for skeletal tissues such as bone, cartilage, hematopoiesis-supporting stroma, and adipocytes [13-15]. BM aspiration and its use in various forms (e.g., regenerative treatments) have gained increased attention in recent years. Fracture repair using autologous BM was perceived to enhance osteogenesis in bone defects and reduce the incidence of delayed bone healing [16-18]. Mandibular fracture union can be assayed clinically, radiographically, and more recently through the use of bone turnover markers (BTMs) [19], such as bone alkaline phosphatase (BAP), osteocalcin (OC), pyridinoline (PYD), and deoxypyridinoline (DPD) [20]. Therefore, the present study investigated the effect of intramedullary injection of autologous BM aspirate on mandibular fracture repair with interdental wire fixation or bone plate fixation in camels based on clinical evaluation, radiographic union score (RUS), and BTMs. To the author's knowledge, this is the first study evaluating the use of BM aspirate injections in mandibular fracture repair in camels.

MATERIALS AND METHODS

Camels

Thirty dromedary camels aged 5–8 years and of both sexes (male, 23; female, 7) were admitted to Veterinary Teaching Hospital, College of Agriculture and Veterinary Medicine, Qassim University. These camels were included in this study on the basis of having clinical and radiographic evidence of recent transverse open mandibular fractures. The study protocol was approved by the Ethics Committee (No. 367) for Animal Research of the Scientific Research Deanship, Qassim University, Saudi Arabia.

Treatment strategies

Based on the treatment regimen used for mandibular fracture repair, camels were randomly assigned into 4 groups: group 1 (n = 10) treated using stainless steel wire fixation, and BM injection at the fracture line, group 2 (n = 10) treated using plate fixation and BM injection at the fracture line, group 3 (n = 5) treated using stainless steel wire fixation and placebo saline injection at the fracture line, and group 4 (n = 5) treated with plate fixation with placebo saline injection at the fracture line. All surgical interventions in groups 1 and 3 were performed by the same surgical team within 20–30 minutes. However, in groups 2 and 4, the surgical procedure was performed by the same surgical team within 60–90 minutes.

Collection of BM aspirate

Camels were placed in a seated position with their forelimbs and hindlimbs tied. The area of tuber coxae was prepared aseptically for the collection of autologous BM aspirate. Approximately 3 mL of lidocaine 2% was infiltrated subcutaneously over the collecting site. The 11-gauge, 10-cm-long BM biopsy needle (Argon Medical Devices; OEM, USA) was inserted in the medial direction perpendicular to the camel's midline through the skin and advanced up to 10 cm until directly contacting the bone. When reaching the bone, the needle was slowly twisted clockwise and counterclockwise to bore into the cortical bone of the tuber coxae. The needle was advanced with a moderate amount of force in a corkscrew (twisting) motion until the needle was well into the marrow cavity. The trocar was removed, and a 20-mL syringe coated with heparin was connected to the needle. Approximately 10-mL BM sample was aspirated. Subsequently, the BM aspirate was transferred into sterile test tubes and centrifuged at 3,200 rpm for 15 min to isolate the BM mononuclear cells and prepare it for use.

Preoperative preparation and sedation

Preoperative antibiotic, penicillin-streptomycin (Pen & Strep; Norbrook Laboratories, UK) at a dose rate of 30,000 IU/kg for the penicillin and 10 mg/kg streptomycin were injected intramuscularly. Flunixine meglumine (Finadyne, Schering-Plough; Intervet Chile Ltda., Netherlands) at 1.1 mg/kg were administered intravenously. Camels were restrained with ropes in the setting position and deeply sedated by intravenous (IV) injection of xylazine HCl (Seton 2%; Laboratorios Calier, S.A., Spain) at dose rate 0.3 mg/kg.

Mandibular fracture repair

Interdental wire and bone marrow (group 1, n = 10)

In this group, the mandibular fracture was fixated by inserting a 0.8-mm-diameter stainless steel wire (IM3 Dental Limited; Duleek, Co., Ireland) between the first and second mandibular premolar cheek teeth or between the second and third cheek teeth, depending on the clinical situation. After passing the wire between the central incisors, the 2-wire edges were twisted together using pliers. Excess wire was trimmed using a wire cutter. However, the remaining edges were twisted toward the body of the incisor teeth. The same technique was then repeated on the other side of the fracture. After thorough fixation of the fracture, intramedullary autologous BM was immediately transplanted via injection to the site of the fracture.

Plate and bone marrow (group 2, n = 10)

A complete reduction of the mandibular fracture in this group was followed by the fixation using a 3.5-mm 10-hole DCP (Ortho-Medical GmbH Implants, Germany). The plate was slightly contoured (overbent) according to the bone curvature, followed by drilling 2.7-mm threaded holes through the bone at points approximately 1 cm from the fracture line, and tapped using 3.5-mm bone taps. Multiple a 3.5-mm cortical screws of proper length were inserted until they began to engage the plate holes. Lastly, transplantation of autologous BM to the fracture site was accomplished via injection.

Interdental wiring alone (group 3, n = 5)

The same technique as described for group 1 was performed on camels in group 3 but with injection of placebo saline (rather than BM aspirate) at the fracture line.

Plate alone (group 4, n = 5)

The same technique as described for group 2 was performed on camels in group 4 but with injection of placebo saline (rather than BM aspirate) at the fracture line.

Postoperative care

Following reduction and fixation of the mandibular fracture, wounds in the oral mucosa were cleaned and sutured. Contaminated wounds were cleaned daily until completely healed, which was accomplished with debridement and lavage with povidone-iodine spray. Preoperative antibiotics and an anti-inflammatory were continued for 5 successive days. All recovering camels were kept tied in the open yard under the same conditions and fed the same type of food. Operated camels were fed milk and barley porridge for 2 weeks, as well as green or dry roughage (according to season) with water free access. Long-term postoperative follow-up of the recovering camels was carried out through clinical assessment, follow-up radiographs, RUS, and BTMs.

Clinical evaluation

Camel owners provided the history for each camel. Each camel was examined carefully to detect and evaluate each fracture. Functional mandible usage of the recovering camels was evaluated in terms of chewing and compared subjectively between groups. Clinical evaluation included, presence pain and crepitus with palpation of the mandible. The presence of long-term complications such as swelling, periosteal reactions, and implant failures were reviewed.

Radiographic evaluation

Radiographic evaluations of the mandibular fracture repairs were conducted at time 0 before surgery and weekly for 12-week post-surgical treatment using MinXray HF 100/30 generator (Toshiba, Japan) with a 70 kVp, 2.0 mAs, and a 70-cm focal film distance. Standard dorsoventral and lateromedial radiographs were obtained for each mandible (**Fig. 1A-I**). All radiographs were subjectively interpreted at different times until complete healing. Each radiograph was interpreted while blinded as to which camels received BM aspirate or saline. Radiographs were evaluated using the modified RUS system based on the presence or absence of a fracture line and callus formation, on radiographs. The score was defined as follows; score 1: visible fracture line visible and absent callus, score 2: visible fracture line visible and visible interrupted callus, score 3: invisible fracture line and visible bridging callus, score 4: no fracture line and no callus. The scores of all cortices were then combined to give a minimum score of 4 (definitely not healed), and a maximum of 10 (completely healed), as described in **Table 1**.

BTMs evaluation

A 16-gauge IV catheter (Mais Co., Saudi Arabia) was placed in the left jugular vein of the recovering camels. Blood samples (5 mL) were collected at time 0 before surgery and weekly thereafter for 12 weeks posttreatment. Blood was collected in clot tubes without

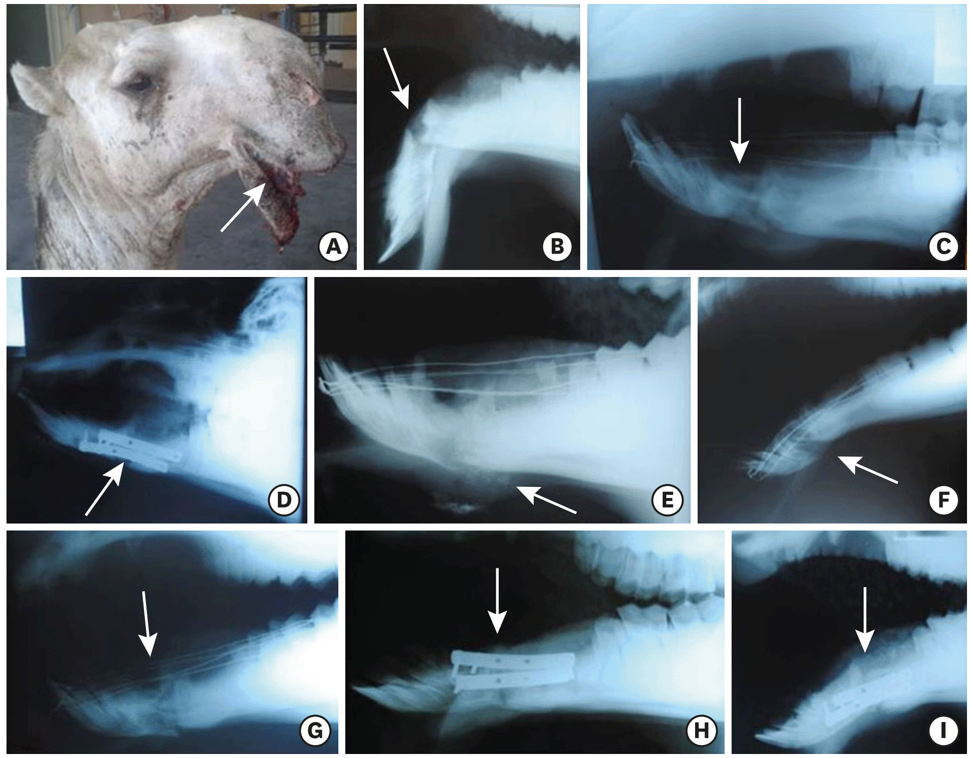

Fig. 1. Clinical photograph (A) and lateral radiograph (B) of complete transverse mandibular fracture in 5 years old camel. Immediate postoperative lateral radiographs of interdental wiring (C) and bone plate fixation (D) for complete mandibular fracture in a 5-years-old camel. (E) Postoperative (day 30) lateral radiographs for interdental wiring fixation + normal saline of a complete mandibular fracture. Sixty days, postoperative lateral radiograph for interdental wiring fixation + bone marrow (F) and interdental wiring + normal saline (G) of a complete mandibular fracture. Postoperative (day 60) lateral radiograph for plate fixation + bone marrow (H) and plate + normal saline (I) of complete mandibular fracture in a 5-years-old camel.

Table 1. The radiographic union scale for subjective assessment of mandibular fracture healing in camels

Radiographic union score	Callus	Fracture line
1	Absent	Visible
2	Interrupted	Visible
3	Bridging	Invisible
4 (Minimum)	Not healed	
10 (Maximum)	Complete healing	

anticoagulant, stored in an ice box, and transported to the laboratory. Sera were separated immediately by centrifugation of the blood at 1,500 × g for 15 min at room temperature and were kept at −20°C until usage. A standard technique using a commercial immunoassay kit (Quidel Corp., USA) was followed to assess the concentration of BTMs, including BAP, PYD, and DPD, according to Al-Sobayil [21,22].

Statistical analysis

Data were analyzed with a commercial statistical software package (SAS version 8, SAS Institute Inc., USA). For scored data, median and range were presented. However, for the continuous data, mean ± SD, were presented. One-way analysis of variance (ANOVA) with *post hoc* Duncan comparison test was used to assess the difference in time of healing among groups. For non-parametric data (radiographic data), Kruskal-Wallis analysis of variance was performed at each time point. However, for the continuous data, repeated measure analysis of variance was used to determine the effect of time and intervention as well as their interaction. For this, Wilks's lambda test was used to assess the interaction between time and treatment. When this test was significant, One-way ANOVA was used at each time point to assess the differences among groups. The Duncan test was used to calculate multiple comparisons to identify which group was statistically different from the rest. Results were considered significant at values of $p < 0.05$.

RESULTS

Clinical findings

According to the history, the main cause of mandibular fracture in this study was biting, with a higher incidence in males (82.06%), particularly during rut season. At the time of presentation, clinical examination of the affected camels revealed the dropped-down incisor teeth in the fractured mandible (**Fig. 1A**). In addition, a slight dullness and depression along with a foul smell arising from the oral cavity and the presence of necrotic debris at the fracture site was noted. The postoperative clinical evaluation revealed a gradual progression in the functional use of the repaired mandible among all groups. The mean ± SD for physical healing time between the treated groups revealed rapid and complete healing, especially in camels treated with wire and BM (56 ± 10 days, 10/10) in comparison to groups 2 (71 ± 14 days, 10/10), 3 (76 ± 13 days, 9/10), and 4 (82 ± 11 days, 8/10), as presented in **Table 2**.

On the 60th postoperative day, all recovering camels in group 1 exhibited full usage of their repaired mandible. An abscess and periosteal reaction were observed at the fracture site in three camels of the third group 30 days postoperatively; however, all remaining camels were stable. In group 4, surgical removal of the implant was performed in one camel, 4 camels required interdental wire in conjunction to the plate (at varied intervals postoperatively), and three cases did not heal.

Radiographic findings

Zero-time radiographic evaluation before surgery of the fractured mandible demonstrated radiolucency, providing evidence of soft-tissue swelling on the lateral view (**Fig. 1B**), with the presence of oblique or transverse fracture lines. Whereas postoperative follow-up radiographs (≤ 6 months) of the camels revealed formation and bridging of the callus to the line of the fracture, thus providing evidence of healing. Changes in the implant or fragment position were reported. Radiographically, proper callus was formed 1-month postoperatively in all studied groups. The formed callus was better at 56 days postoperatively in group 1 than at other groups (**Fig. 1F**). The periosteal reaction was observed in three camels in group 3, 30 days after surgery (**Fig. 1E**).

The RUS were statistically similar in all groups until the 35th day postoperatively ($p = 0.126$). However, on the postoperative day 56 and 63, group 1 had a statistically significant higher

Table 2. Treatment outcomes of mandibular fractures in camels, by group

Group	Time (day)	Recovery rate
G1 (n = 10)	56 ± 10^{a}	$10/10^{a}$
G2 (n = 10)	71 ± 14^{b}	$10/10^{a}$
G3 (n = 5)	76 ± 13^{b}	$9/10^{b}$
G4 (n = 5)	82 ± 11^{c}	$8/10^{c}$

Camels were randomly assigned into 4 groups: G1, interdental wire fixation and bone marrow; G2, plate fixation and bone marrow; G3, interdental wiring fixation alone; G4, plate fixation alone.
$^{a-c}$Mean ± SD different superscript letters at the same column are significantly different at $p < 0.05$.

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Table 3. The median and range of the radiographic union score for mandibular fractures in camels, by group

Time post-treatment	Radiographic score			
	Group 1	Group 2	Group 3	Group 4
7th day	3 (1–4)	3 (1–4)	3 (1–4)	3 (1–4)
14th day	2.5 (2–3)	3 (2–3)	3 (1–4)	3 (1–4)
21th day	2.5 (1–3)	2.5 (2–3)	3 (2–3)	3 (2–3)
28th day	2 (1–3)	2.5 (2–3)	2.5 (2–3)	3 (1–3)
35th day	1.5 (0–3)	2 (1–3)	2.5 (1–3)	2.5 (2–3)
42th day	1 (0–2)	1.5 (2–3)	2 (1–3)	2.5 (1–3)
49th day	0.5 (1–2)	1 (1–3)	1.5 (1–2)	2 (1–2)
56th day	0 (0–1)[a]	1 (1–2)	1 (1–1)	1.5 (1–1)
63th day	0 (0–0)[a]	0.5 (1–1)	0.5 (0–1)	1 (0–1)
70th day	0 (0–0)[a]	0 (0–0)[b]	0.5 (0–1)	0.5 (0–1)
77th day	0 (0–0)[a]	0 (0–0)[b]	0 (0–1)[c]	0.5 (0–0)[d]
84th day	0 (0–0)[a]	0 (0–0)[b]	0 (0–0)[c]	0 (0–0)[d]

Camels were randomly assigned into 4 groups: G1, interdental wire fixation and bone marrow; G2, plate fixation and bone marrow; G3, interdental wiring fixation alone; G4, plate fixation alone.
[a-d]Medians and ranges with different superscript letters at the same column are significantly different at $p < 0.05$.

RUS and visible callus formation ($p = 0.001$ and $p = 0.000$, respectively) than the other treatment groups (**Table 3**).

BTMs findings

BAP levels were significantly elevated in group 1 starting from the second week to the sixth week postoperatively and then dropped to normal levels. In group 2, BAP levels were significantly elevated from the fourth to seventh week. BAP elevation in groups 3 and 4 started at the sixth follow-up week but was not significantly different from 0 time before surgery (**Fig. 2A**). OC levels were significantly increased in group 1 starting from week 5 and in group 2 starting from week 6 and remained until completely healed. In groups 3 and 4, however, OC elevation started from week 9 postoperatively and continued until the end of the study (**Fig. 2B**).

PYD levels were significantly elevated in group 1 starting from the second to third week. In group 2, PYD levels were significantly elevated from the second to fifth postoperative week. Also, PYD levels in group 3 were elevated between the second and eighth week. PYD concentration in group 4 was elevated from the 2nd to 10th postoperative week (**Fig. 2C**). DPD levels were significantly elevated in group 1 by the first to third week and in group 2 from the second to sixth week. In group 3, DPD levels were increased between the second and seventh postoperative week. However, DPD in group 4 were increased from the third to tenth week postoperatively (**Fig. 2D**). Descriptive details of the BTMs are presented in **Fig. 2**.

DISCUSSION

Dromedary camels play a significant role in the livestock agricultural economy in the Horn of Africa, Middle East, and South Asia. Every year, a large number of valuable camels suffer mandibular fractures, resulting in great economic loss [10,23]. Often as a result of biting by sexually excited camel bulls during rut season, in this study, the number of males (n = 23, 82.06%) with mandibular fractures was more than females (n = 7, 17.94%). In most studies of the mandible of camels, fractures were presented in the interdental space, just cranial or caudal to the first premolars due to the presence of rostral mental foramina and canine alveoli at this region, as it represents the weakest point of the mandible. These findings are in agreement with Ahmed [3] and Hanuman et al. [9].

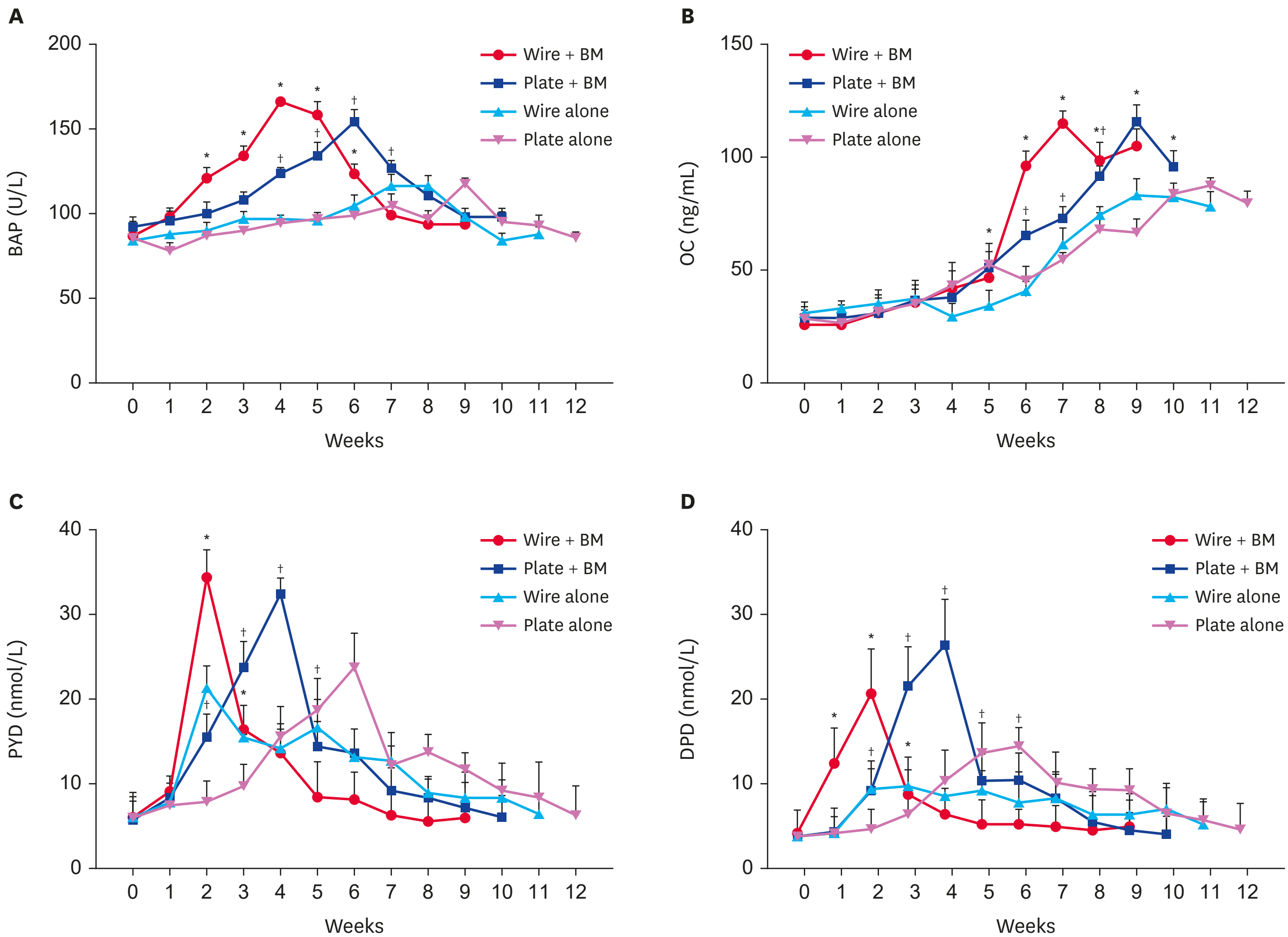

Fig. 2. (A) BAP, (B) OC, (C) PYD, and (D) DPD levels for camels with mandibular fractures repaired by wire and plate fixation with and without BM.
BAP, bone alkaline phosphatase; OC, osteocalcin; PYD, pyridinoline; DPD, deoxypyridinoline; BM, bone marrow.
There was a significant increase of the levels of BAP, OC, PYD and DPD in groups 1 (*) and 2 (†) compared to the control group.

Interdental wiring and bone plate fixation were used in camels and represent traditional techniques for mandibular fracture repair. Unfortunately, improper healing may occur due to a variety of factors related to the animal, environment, and fixation tool [2-4]. Therefore, this study was conducted to overcome these complications by investigating the applicability and feasibility of autologous BM aspirate as adjuvant treatment for mandibular fracture repair with interdental wire or bone plate fixation.

Various sites for BM aspirate have been described in animals including the ribs, sternum, tuber coxae, fourth tarsal bone, and third metacarpal bone [24]. However, because the most suitable sitting position for camels is sternal recumbency (as it allows proper restraint of the animal), BM aspiration from the sternum would be more difficult because of its direct contact with the ground and corresponding risk of sample contamination. Based on this risk, we prioritized for the tuber coxae for BM harvesting. This anatomical site is easily accessible in camels with minimal overlying soft tissue, and this allows for easy collection, better safety, low wound morbidity, and less discomfort for the camel.

The effect of BM on bone repair may not be due solely due to its osteogenic function but to the synergistic effects from osteoprogenitor cells derived from BM. In addition, its capacity to secrete trophic factors enhances the repair process via improvements in vascularization, the inhibition of cell death, and modulation of the immune response [17,25]. In this study, autologous BM injection at the site of mandibular fracture provided improved results as evidenced by a proper callus formation occurring most timely in group 1 when compared to the nature and duration of the formed callus in camels of other groups. These findings were in agreement with Kitoh et al. [19], Chapman [26], and Hernigou et al. [27].

The results of this study revealed that interdental wire fixation with BM injection provided excellent conditions for rapid and proper healing of mandibular fractures (56 ± 10, 10/10) in comparison to plate fixation with BM injection (71 ± 14, 10/10), interdental wiring alone (76 ± 13, 9/10), and plate alone (82 ± 11, 8/10). This could be explained by the osteogenic properties of the BM that enhance and improve the fracture repair, in conjunction with the biomechanical properties of the interdental wiring providing good stability without direct contact with the fracture site or damage to the tooth roots as occurred in plate fixation. Moreover, this technique can be performed with minimal cost and time required for fracture fixation and without the need for special surgical instruments as with the use of bone plates. These findings were in accordance with Ahmed [3] and Nuss et al. [28].

On the 56th postoperative day, radiological appearance of the injected group demonstrated full maturation of new bone in the fracture line and with proper callus formation, in comparison to the other treatment groups. The RUS were statistically similar in all groups from the 35th postoperative day ($p = 0.126$), when group 1 exhibited significantly higher RUS and visible callus formation ($p = 0.001$ and $p = 0.000$, respectively), to the 56th postoperative day. This result objectively demonstrates the positive effect of BM injection on the radiologic properties of newly formed bone at the mandibular fracture line.

BTMs have been considered as an auxiliary diagnostic or prognostic technique for monitoring fracture healing. Measurement of BTMs during the fracture healing process could enhance the accuracy of bone healing stage assessment, allow for earlier intervention, and improve patient care [29,30]. Our results revealed significant elevations of the levels of BAP, OC, PYD, and DPD in group 1 and 2 compared to the control groups. This could be related to the active synthesis and maturation stage of the bone extracellular matrix by osteoblasts stimulated during the fracture healing process.

Although radiography is the gold standard technique for the evaluation of fracture healing, it may not be reliable for the rapid detection of bone repair complications. Early diagnosis of such complications could prevent prolonged patient suffering, disability, and economic loss [18,19]. Great efforts have been made toward the development of new technologies for improving precision in diagnosing complications following bone fractures [29,30]. Therefore, the collaboration system between the clinical evaluation, RUS, and BTMs used in this study provides a promising and simple noninvasive tool for subjective assessment and outcome prediction for mandibular fracture repair in camels. These findings were in concordant with El-Shafaey et al. [31], Ali et al. [32], and Sousa et al. [33].

The limitation of the present study was the small sample size of studied camels, which does not allow existing conclusion. Moreover, the evaluation of BM for improving mandibular fractures repair was presented alone. Comparative randomized clinical study with other biological materials improving repair of the mandibular fracture are warranted to get precise conclusion about the clinical relevance of BM in camels.

In conclusion, BM aspirate has a promising and beneficial osteogenic effect on mandibular fracture repair in camels, specifically when used in conjunction with interdental wire fixation. Further research is required to evaluate the efficacy and limitations of BM injection relative to other biological adjuvants for repairing mandibular fractures in camels.

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