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ORIGINAL ARTICLE

Potential Effect of Platelet-Rich Fibrin and Chitosan on Healing of Surgically Induced Lacerated Superficial Digital Flexor Tendon in Donkeys

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Abstract

OBJECTIVE: To investigate the effects of platelet-rich fibrin (PRF) and chitosan (Ch) on the healing of superficial digital flexor tendon (SDFT) defects in a donkey model.
DESIGN: Randomized experimental study.
ANIMALS: Eighteen clinically healthy male donkeys. A full-thickness defect of the SDFT was performed at the mid-metatarsus, and tenorrhaphy was performed, leaving a 1 cm defect. The animals were allocated into three groups (six animals/group). Group I (control group): no biomaterials were added; group II (PRF group): the SDFT gap was filled with autogenous PRF; group III (PRF/Ch group): a combination of PRF and chitosan was used to fill the SDFT gap. Ultrasonographic examinations were performed at 1, 2, and 3 months postoperatively, and the imaging characteristics were compared at each time point.
RESULTS: The PRF and PRF/Ch groups showed significant ($P > 0.00$) improvements in tendon echogenicity, fiber alignment, and thickness compared with the control group. In the PRF-and PRF/Ch-treated groups, SDFT showed a well oriented tendon fibers with normal thickness and normal crescent shape.
CONCLUSION AND CLINICAL RELEVANCE: Based on tendon echogenicity, fiber alignment, position, and thickness, PRF and chitosan can improve tendon healing and could provide a new bioscaffold-based strategy for SDFT regeneration in donkeys.

Keywords: Chitosan, Platelet rich fibrin, Superficial digital flexor tendon

1. Introduction

Tendons are brilliant white strong fibro-elastic tissues with a small number of cells and a rich extracellular matrix [1]. Tendon lacerations are serious injuries in horses because of the loss of biomechanical function of the tendon, slow return of tendon strength, immediate strenuous loading demanded by the equine patient, and scarring complications [2]. It is caused by external trauma resulting in laceration or disruption of the flexors and extensor tendons [3] or intrinsic trauma secondary to continuous stress (self-inflicted wounds) of tensile loading, resulting in tendonitis or bowed tendon [4,5].

When lacerations involve tendons, the treatment and prognosis of return to use are variable. The treatment of flexor tendon laceration involves immobilization with or without tendon suturing. Limb braces, board bandage splints, and castings were used. With each approach, recommendations for the duration of immobilization have been made [6].

Platelet-rich fibrin (PRF) is a concentrated platelet on a fibrin membrane containing important growth

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factors and has been used to improve the healing of hyper granulating wounds [7] and osteochondral defects [8] because the fibrin matrix acts as a scaffold to enhance cell migration and proliferation [9].

Chitosan is a linear, semi-crystalline polysaccharide that has numerous properties, such as biocompatibility and biodegradability, making it suitable for use in different biomedical fields, especially tissue engineering. Chitosan prevents adhesion during tendon repair [10,11].

Ultrasonography is one of the most accurate, well-tolerated, and noninvasive tools to diagnose the type of injury, quantify its severity, follow-up the healing process, and establish the prognosis. The key for clinicians is to produce images of the highest quality to maximize the diagnostic information obtained [12]. El-Husseiny [13] found that ultrasonographic examination of ruptured digital flexor tendons revealed the presence of two echogenic ruptured tendinous segments, and the cut area appeared anechoic in between with peritendinous anechoic fluid. This core lesion occupied about 65% of the superficial digital flexor tendon (SDFT) in the cross section, while the longitudinal view showed a lack of fiber arrangement. This fiber tearing could be multiple and showed a hypoechoic to anechoic area with slight loss of fiber alignment at the dorsal aspect and center of the SDFT [14]. The present study aimed to assess the efficacy of PRF and chitosan (Ch) in SDFT defect healing in donkeys using ultrasonographic examinations.

2. Material and methods

2.1. Animals

The present study was carried out on 18 clinically healthy male donkeys aged 24.5 ± 3.4 months old and weighing 126.5 ± 12.1 kg. Two weeks before the experiment, donkeys were housed in separate hygienic conditions, fed on a balanced ration, and administered the anthelmintic drug doramectin (Dectomax, Zoetis, United States) at a dose rate of 1 ml/50 kg subcutaneously. The present study was approved by the Medical Research Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, code No: M/21. All animals were evaluated clinically and subjected to ultrasonographic examination of the SDFT to ensure their liability for the experiment.

2.2. Experimental study

In all donkeys (n = 18), the SDFT of the right hind limb was severed at the middle of the metatarsal region, and tenorrhaphy was performed using a bunnel suture pattern, leaving a full-thickness defect of 1 cm in length. Donkeys were randomly divided into three groups (six animals each) based on the biomaterial scaffold used to fill the defects.

(1) Group I (Control group): no biomaterials were added, and the gap was left empty.
(2) Group II (PRF group): the SDFT gap was filled with autogenous PRF of equal size.
(3) Group III (PRF/chitosan group) (PRF/CH group): a combination of autogenous PRF and chitosan was used to fill the SDFT gap.

2.3. Preparation of PRF

PRF was prepared according to the method described by Choukroun et al. [15]. Briefly, 4 ml of whole blood in a plain tube was immediately centrifuged at 3000 rpm for 15 min using a laboratory centrifuge until separation into three distinct layers: the upper layer consisted of platelet-poor plasma, the PRF clot in the middle layer, and RBCs at the bottom of the test tube. This clot was carefully placed in the tendon defect (Fig. 1).

2.4. Preparation of chitosan

The aqueous solution (1%) of glacial acetic acid was prepared, then, chitosan solution (2.00% w/v) was prepared by adding 2.00 g chitosan to 100 mL acetic acid (1%) while stirring on a magnetic stirrer hot plate (Fig. 2). The solution was stirred at low temperature (50 °C) for 1 h. The resultant chitosan solution was filtered through No. 3 filter paper to remove any undissolved particles. To overcome the fragility of chitosan, glycerol was added to 30.00% of the total solid weight in the solution [16].

2.5. Surgical procedures

2.5.1. Preoperative preparation

The operated limbs were aseptically prepared one day before the operation. Food and water were withheld for 8−12 h preoperatively. Moreover, a broad-spectrum antibiotic, penicillin and streptomycin (pen and strept, Norbrook, UK), was injected intramuscularly 2 h before surgery at a dose of 8 mg/kg procaine penicillin with 10 mg/kg dihydrostreptomycin equivalent to 1 ml/25 kg body weight.

2.5.2. Anesthetic protocol

A 14-gauge IV catheter was placed in the jugular vein. The animals were premedicated with acepromazine

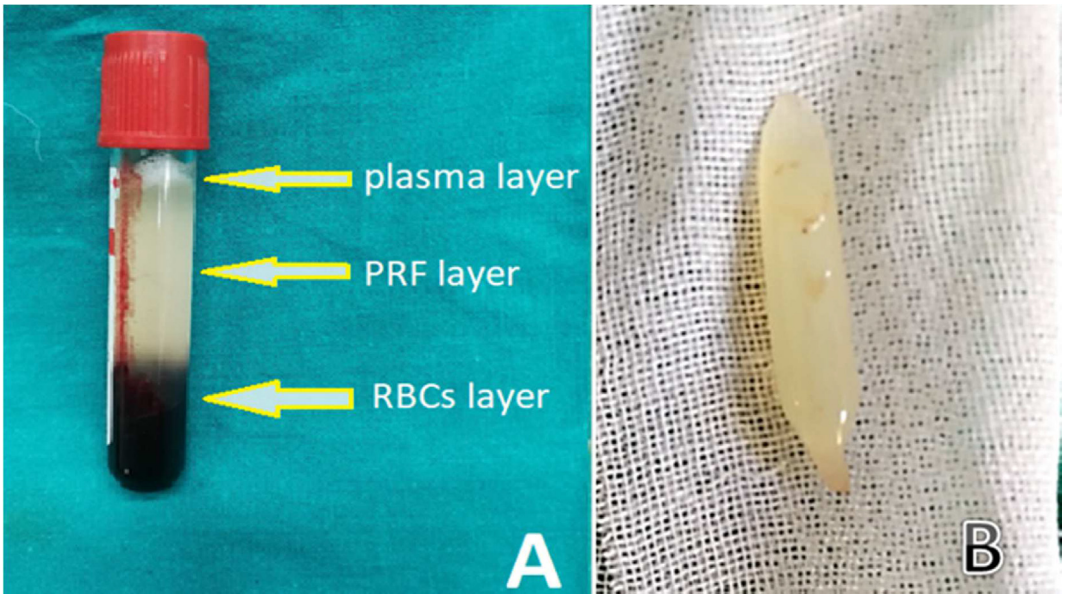

Fig. 1. Showing plain tube contains the blood after centrifugation (A) and platelet-rich fibringel graft (B).

maleate at a dose of 0.05 mg/kg (Castran, 15 mg/mL; Interchemie, Holland) and xylazine Hcl at a dose of 1.1 mg/kg (Xyla-Ject, 2%; Adwia, Egypt) with 20 min interval. General anesthesia was induced using IV injection of propofol 1% (Diprivan 1%, Corden Pharma, Italy) at a dose of 2 mg/kg body weight. The donkeys were secured in lateral recumbency, with the intended right hind limb positioned uppermost in an extension position.

Intravenous regional anesthesia was accomplished after Samy et al. [17] using a slow infusion of 20 ml of lidocaine hydrochloride (Debocaine 2%; Sigmatec Pharmaceutical Industries Co, Egypt) in the common dorsal digital vein. During the operation, the depth of anesthesia was assessed, and the maintenance dose of propofol was (0.2 mg/kgbwt/min).

2.5.3. Surgical procedure

A 7 cm linear skin incision was made over the plantar aspect of the mid-cannon region. After careful blunt dissection and approaching of the paratenon, a sharp longitudinal incision was made to expose and transect the SDFT. The ends of the transected tendon were sutured with a Bunnell suture pattern using polypropylene size 1 (Egyprolene, TAISIER-MED, Egypt) leaving a 1 cm defect. The defect was either left empty without scaffolds or filled with PRF (PRF group), or PRF and chitosan (PRF/

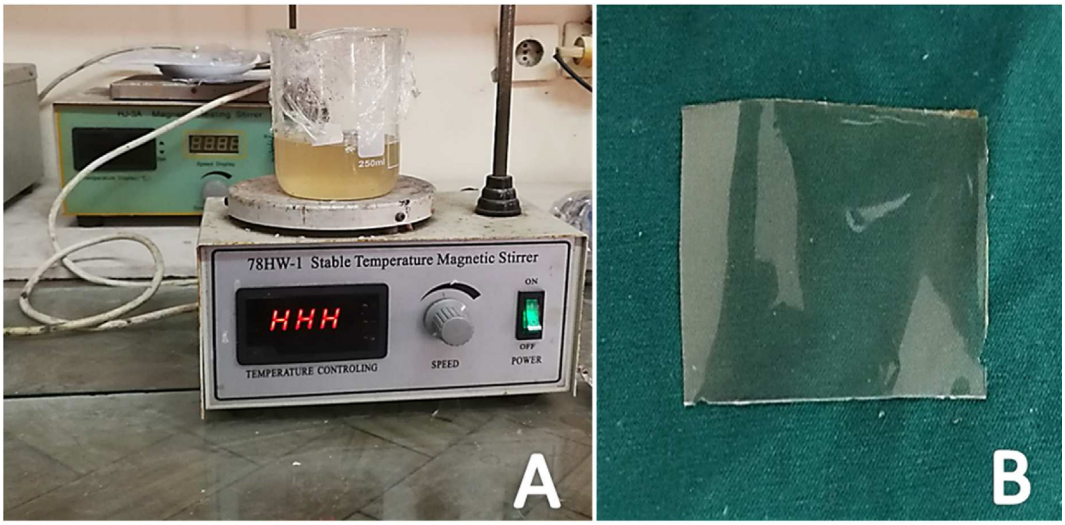

Fig. 2. Showing magnetic stirrer-hot plate (A) that used in preparation of chitosan (B).

chitosan group). Both the paratenon and subcutaneous tissues were closed separately with a simple continuous suture pattern using polyglycolic Acid No 0 (Egysorb, TAISIER-MED, Egypt). Skin closure was accomplished using an interrupted cross-mattress suture pattern with polypropylene No. 1. Finally, the surgical wound was covered with sterile nonadherent medicated pads, and the limb was immobilized using a distal limb fiberglass cast (Optima cast, Enterprise Co, Korea) For 8 weeks with maintaining the fetlock angle in semi-flexion position after that an extended heel shoe was used till the end of the experiment. A window was made in the fiberglass cast facing the operative wound for daily wound dressing, and the medicated sterile gauze pads used to cover the wound were changed regularly until complete healing of the wound and removal of skin sutures (Fig. 3).

2.5.4. Postoperative management

Preoperative antibiotics were continued for 5 days, and Anti-inflammatory Flunixin meglumine at a dose rate of 1.1 mg/kg (Flamicure 5%, Pharma Swede, Egypt) for 5 successive days and antitetanic serum 1500 IU/animal were injected on the day of surgery. The animals were subjected to controlled hand-walk exercises for 10 min daily after the first month postoperatively.

2.6. Ultrasonographic evaluation

Ultrasonographic scanning was performed at 1, 2, and 3 months postoperatively in both the longitudinal and transverse planes Using a 7-12-MHz linear transducer ultrasound system (CHISON Digital Color Doppler Ultrasound system, iVis 60 EXPERT VET; CHISON Medical imaging Co., Ltd, China) for assessment of tendon healing SDFT echogenicity, alignment, thickness, shape, and position.

3. Results

3.1. At one month postoperative (PO)

The superficial digital flexor tendon of the control group showed complete anechoic content at the site

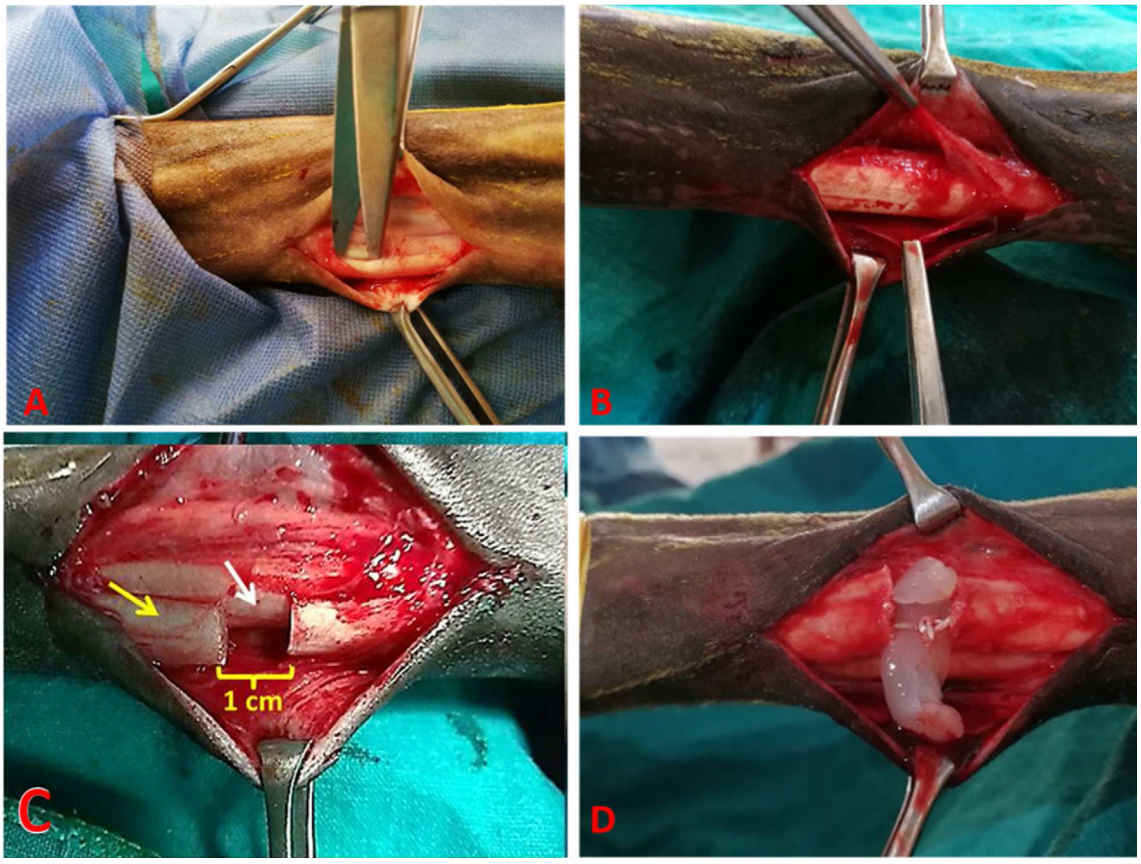

Fig. 3. A: Showing the skin incision over the mid plantar aspect of the metatarsus. B: Showing superficial digital flexor tendon after paratenon incision. C: Showing the superficial digital flexor tendon with 1 cm defect. D: Showing the superficial digital flexor tendon defect filled with a platelet-rich fibrin.

of segmental defect with retraction of tendon fibers above and below the segmental defect and a marked increase in thickness, as shown in the longitudinal section. In the cross-sectional image, the area of interest showed gradual filling with the presence of multiple anechoic areas (Fig. 4).

In the PRF group, the SDFT showed partial filling of the defect by hypoechoic dots with a large anechoic center and a marked increase in SDFT thickness, while in the cross-sectional image, the defect site was filled by disoriented displaced hypoechoic tissue with minimal areas of decreased echogenicity (Fig. 5).

The superficial digital flexor tendon of the PRF/Ch group showed the presence of complete anechoic content at the site of the segmental defect with a marked increase in thickness, as shown in the longitudinal section, while in the cross-sectional image, the defect site was almost filled by hypoechoic dots with multiple anechoic areas (Fig. 6).

3.2. At 2-month postoperative

SDFT of the control showed the area of anechoic content still present and represents the tendon defect and still marked increase in thickness as shown by longitudinal section, while in cross section, the defect site was filled by irregularly shaped tissue with intratendinous edema (Fig. 7).

In the PRF group, the SDFT showed well-formed and oriented tendon fibers with minimal hypoechoic areas and anastomosis between the newly formed tissue and the healthy tendon (hyperechoic line) with a mild increase in thickness, as shown in the longitudinal section. The cross-sectional image shows newly formed and oriented displaced tendon fibers with a normal crescent shape (Fig. 8).

The SDFT of the PRF/Ch group showed diffuse intratendinous edema in well-formed and oriented tendon fibers and hardly any demarcation between injured and healthy tissues with a mild increase in thickness, as shown in the longitudinal section. The cross-sectional image showed well-formed and oriented tendon fibers with normal crescent shape, and faint signs of hypoechoic area remained (Fig. 9).

3.3. At third-month postoperative

Superficial digital flexor tendon of the control group showed disoriented tendon fibers with subcutaneous reaction represented by anechoic fluid, and the area of interest still thickened while the other segment retained nearly normal thickness, while in cross-section SDFT, enlarged irregularly shaped tendons appeared (Fig. 10).

In the PRF group, the SDFT showed well-formed and oriented tendon fibers with nearly normal thickness, as shown in the longitudinal section. The cross-sectional image shows well-formed and oriented displaced tendon fibers with a normal crescent shape (Fig. 11).

The superficial digital flexor tendon of the PRF/Ch group showed well-formed and oriented tendon fibers with minimal hypoechoic areas and a mild increase in thickness, as shown in the longitudinal section. The cross-sectional image shows a well-formed and oriented displaced enlarged tendon with a normal crescent shape (Fig. 12).

4. Discussion

The present study aimed to assess the efficacy of PRF and Chitosan in treating surgically induced SDFT lacerations in donkeys. The findings of this study revealed the positive effect of PRF and/or chitosan on the healing of SDFT in donkeys. Their effects gradually increased from the first to the third month based on ultrasonographic assessment [18,19].

Ultrasonography is one of the most accurate, well-tolerated, and noninvasive tools to diagnose and characterize the type of injury, quantify its severity, follow-up the healing process, and establish the prognosis [12].

In the present study, SDFT was subjected to tenectomy, where ~1 cm of its full thickness was removed to form a gap of 1 cm between the cut ends because the major clinical problem of tendon laceration encountered during surgical management is the formation of a gap between the cut ends, which prevents bringing together the cut ends by simple tenorrhaphy [20].

Ultrasonographic examination in the control group, which was demonstrated at 30 days postoperation, showed the presence of anechoic content at the site of segmental defect without orientation of the tendon fiber and a marked increase in thickness. At 60 days, the sonogram showed disorientation of the fibers with irregular fibrous tissue and presence of intratendinous edema. At 90 days, well-formed and disoriented tendon fibers with subcutaneous reactions represented by anechoic fluids were still thickened. These results are in agreement with those of El-Shafaey [21].

In the PRF-treated group, ultrasonography at 30 days showed partial filling of the defect by more echogenic dots denoting repair of SDFT with large anechoic content and marked increase in SDFT thickness. These findings were consistent with those reported by Mostafa [22]. At 2 months until the end

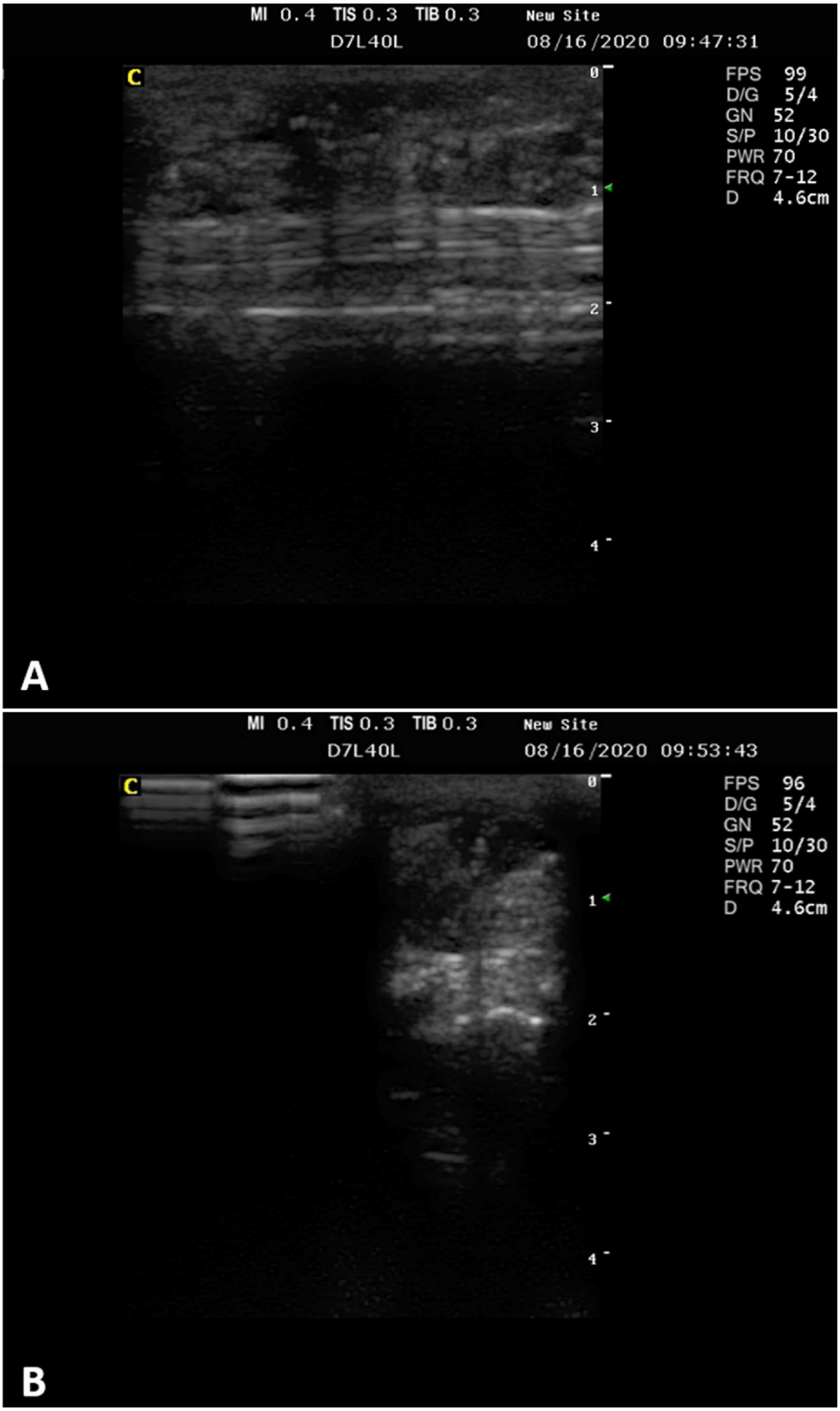

Fig. 4. Ultrasonographic appearance of superficial digital flexor tendon at one month (control group). A longitudinal sonogram showing marked increase in superficial digital flexor tendon thickness with increase in the anechoic content at the site of segmental defect. The hyperechoic dots in the anechoic area represents the polypropylene suture material) with distal shadowing B Transverse sonogram showing the defect site gradually filling with presence of multiple anechoic areas.

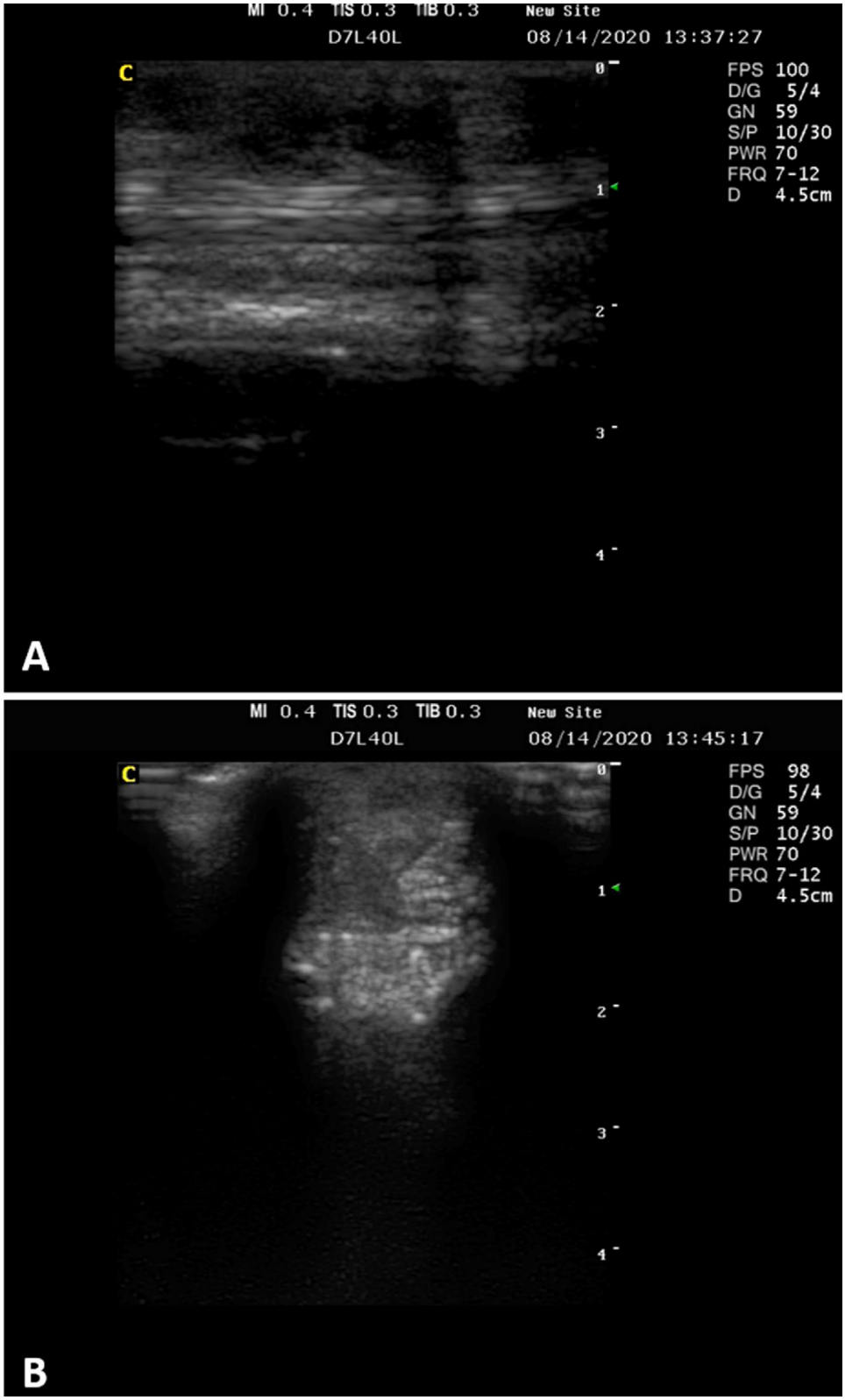

Fig. 5. Ultrasonographic appearance of superficial digital flexor tendon at one month (platelet-rich fibrin group). A Longitudinal sonogram showing marked increase in superficial digital flexor tendon thickness with increase in the anechoic content at the site of segmental defect. B Transverse sonogram showing the defect site completely filled with disoriented displaced hyperechoic dots with presence of minimal areas with decreased echogenicity.

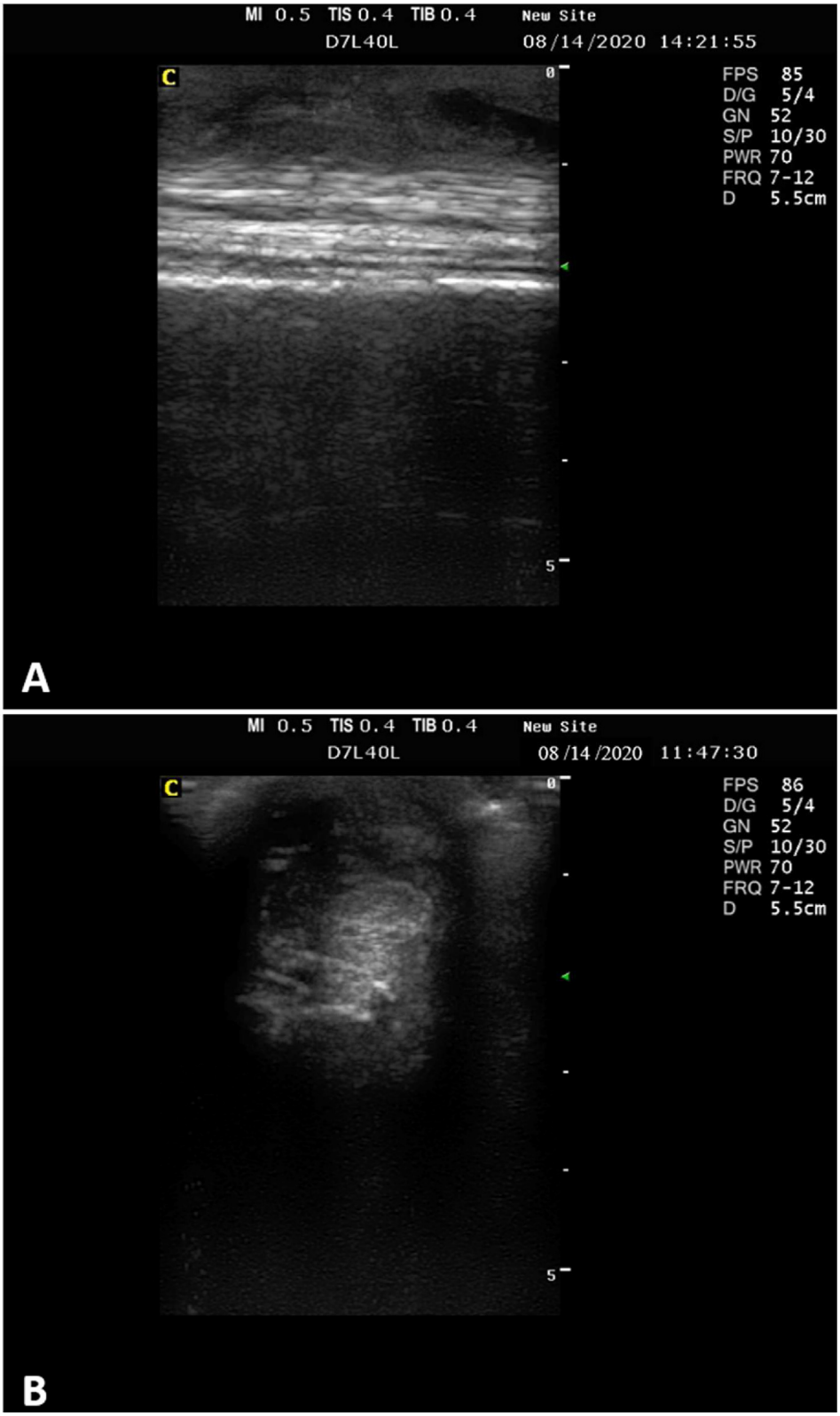

Fig. 6. Ultrasonographic appearance of superficial digital flexor tendon at one month (platelet-rich fibrin/chitosan group). A Longitudinal sonogram showing marked increase in superficial digital flexor tendon thickness with increase in the anechoic content at the area of interest. B Transverse sonogram showing the defect site almost filled by hypoechoic dots with presence of multiple areas with decreased echogenicity.

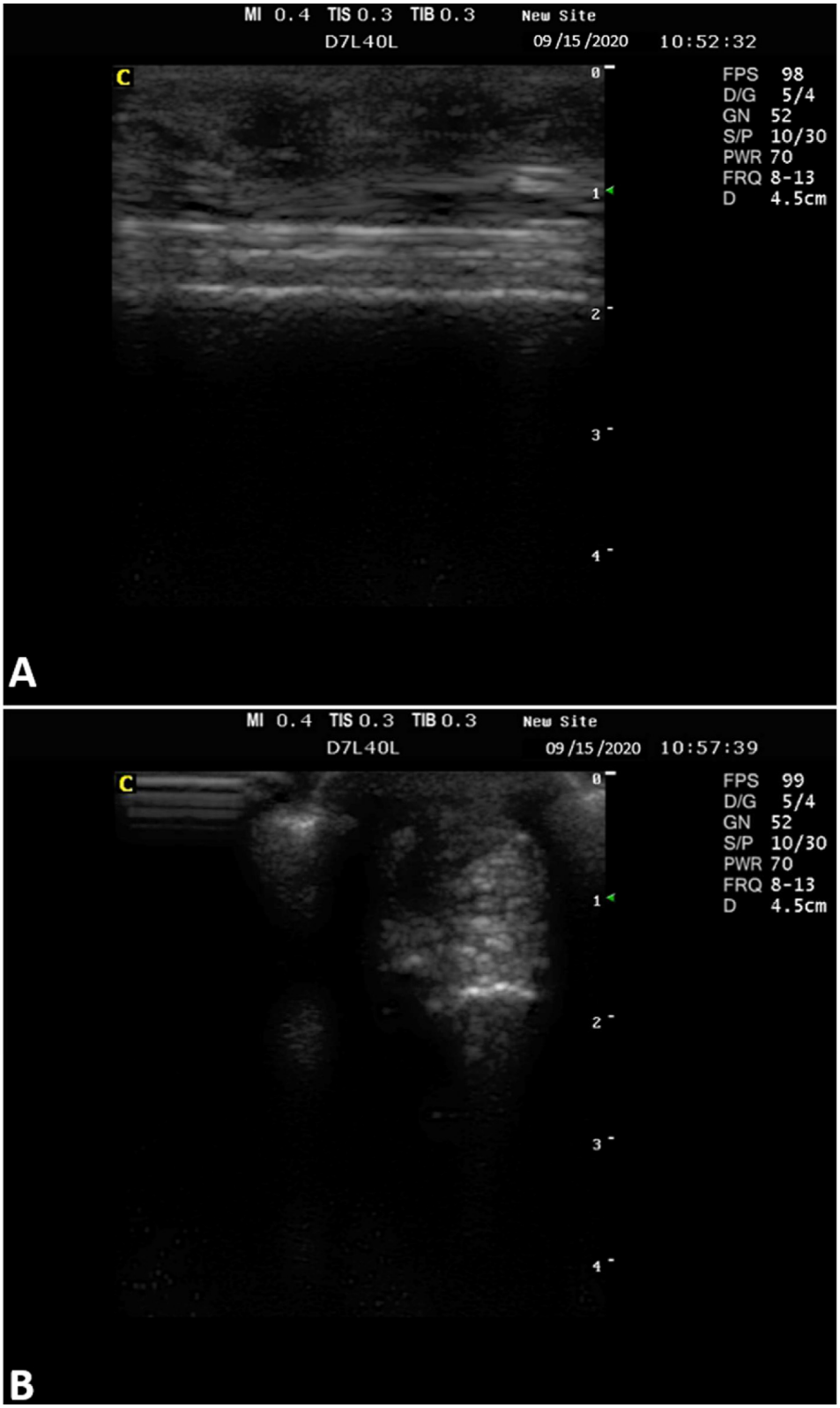

Fig. 7. Ultrasonographic appearance of superficial digital flexor tendon at two months (control group). A Longitudinal sonogram showing increase anechoic content at the area of interest with still increase in thickness. B Transverse sonogram showing irregular shaped displaced superficial digital flexor tendon with intratendinous edema.

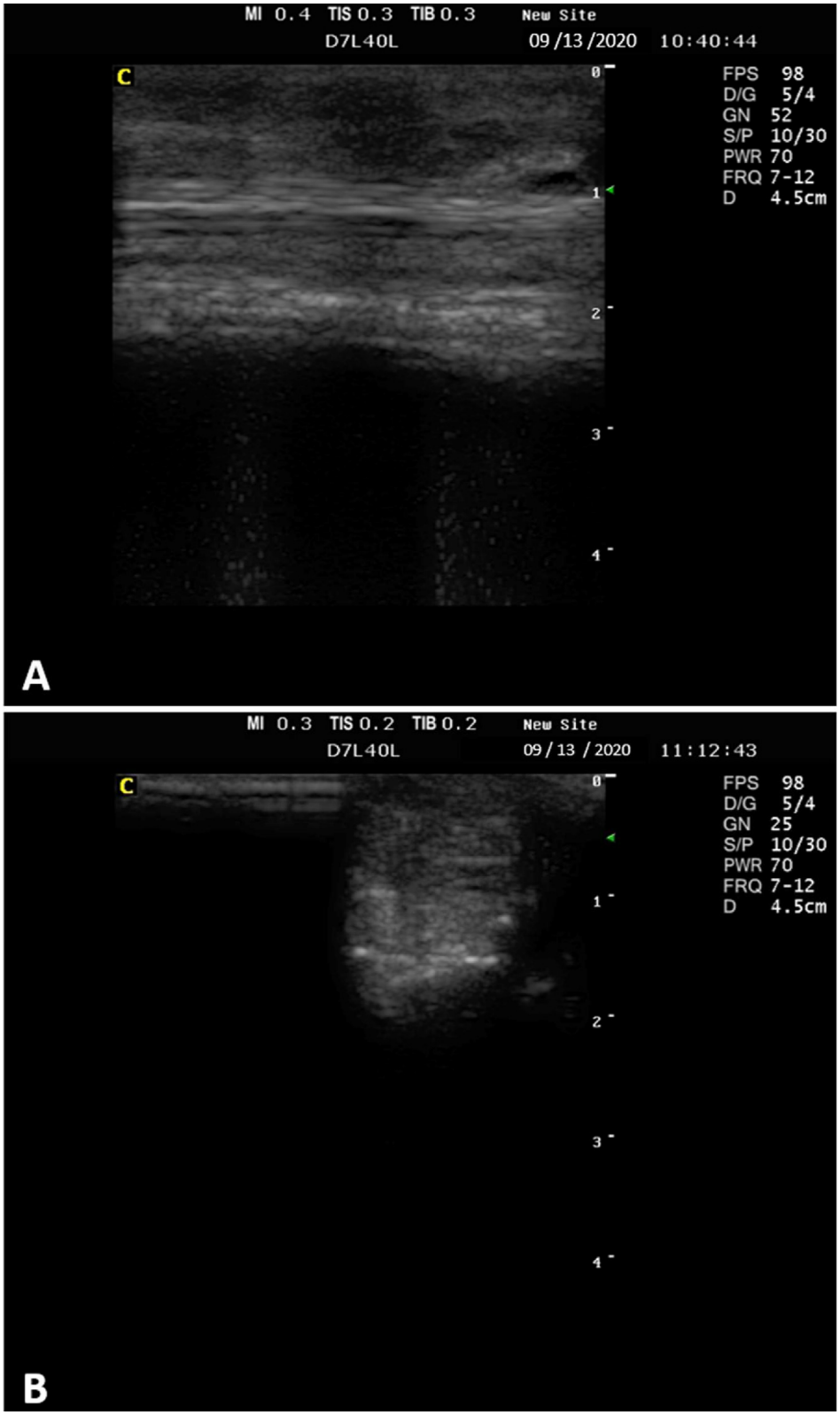

Fig. 8. Ultrasonographic appearance of superficial digital flexor tendon at two months (platelet-rich fibrin group). A Longitudinal sonogram Showing well-formed and oriented tendon fiber with minimal hypoechoic areas and anastomosis between the newly formed tissue and healthy tendon (hyperechoic line). B Transverse sonogram showing newly formed oriented displaced tendon with normal crescent shape.

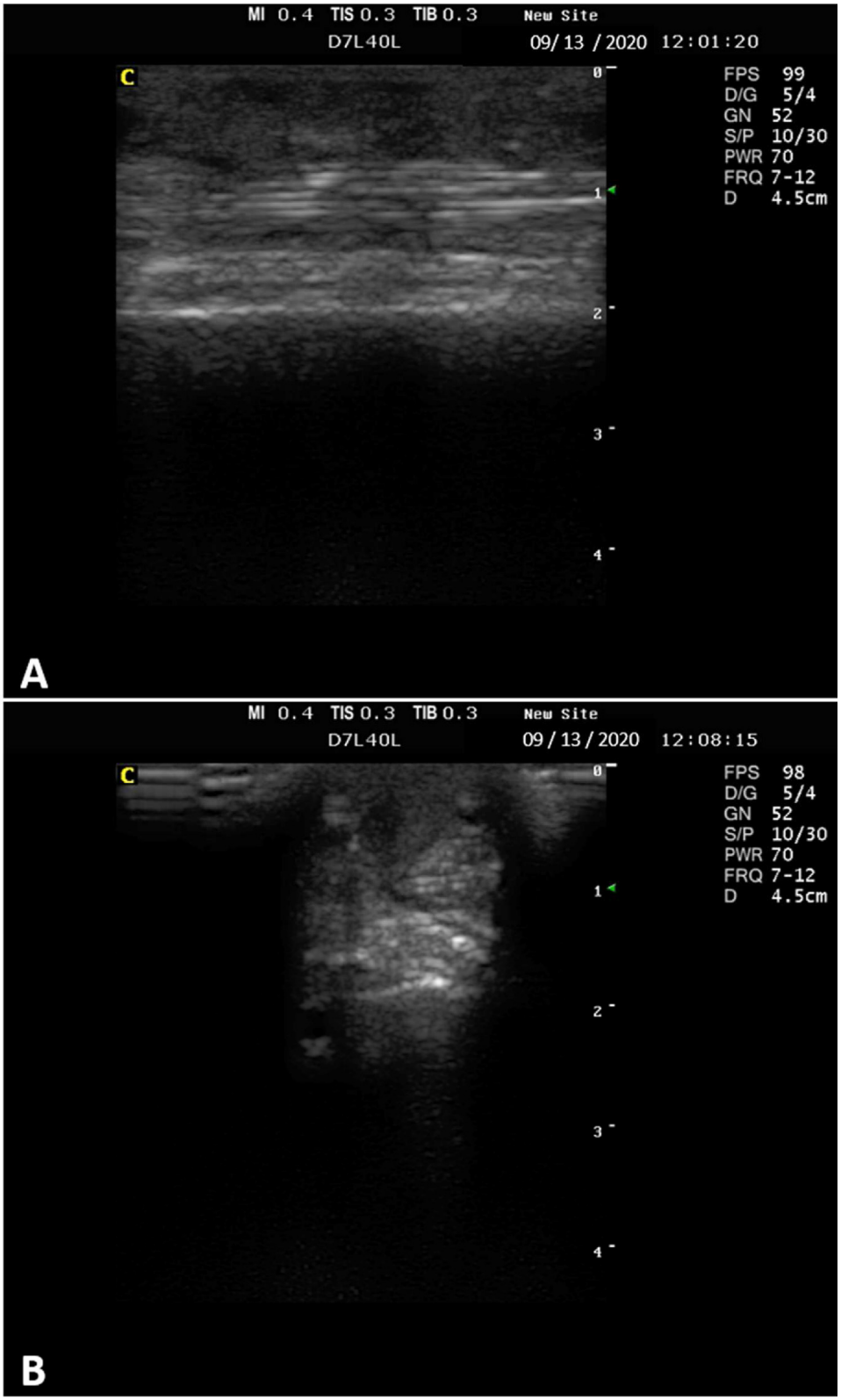

Fig. 9. Ultrasonographic appearance of superficial digital flexor tendon at two months (platelet-rich fibrin/chitosan group). A Longitudinal sonogram Showing well-formed and oriented tendon fiber and hardly any demarcation between injured and healthy tissue. B Transverse sonogram showing well-formed oriented tendon fiber with normal crescent shape faint signs of hypoechoic area remain.

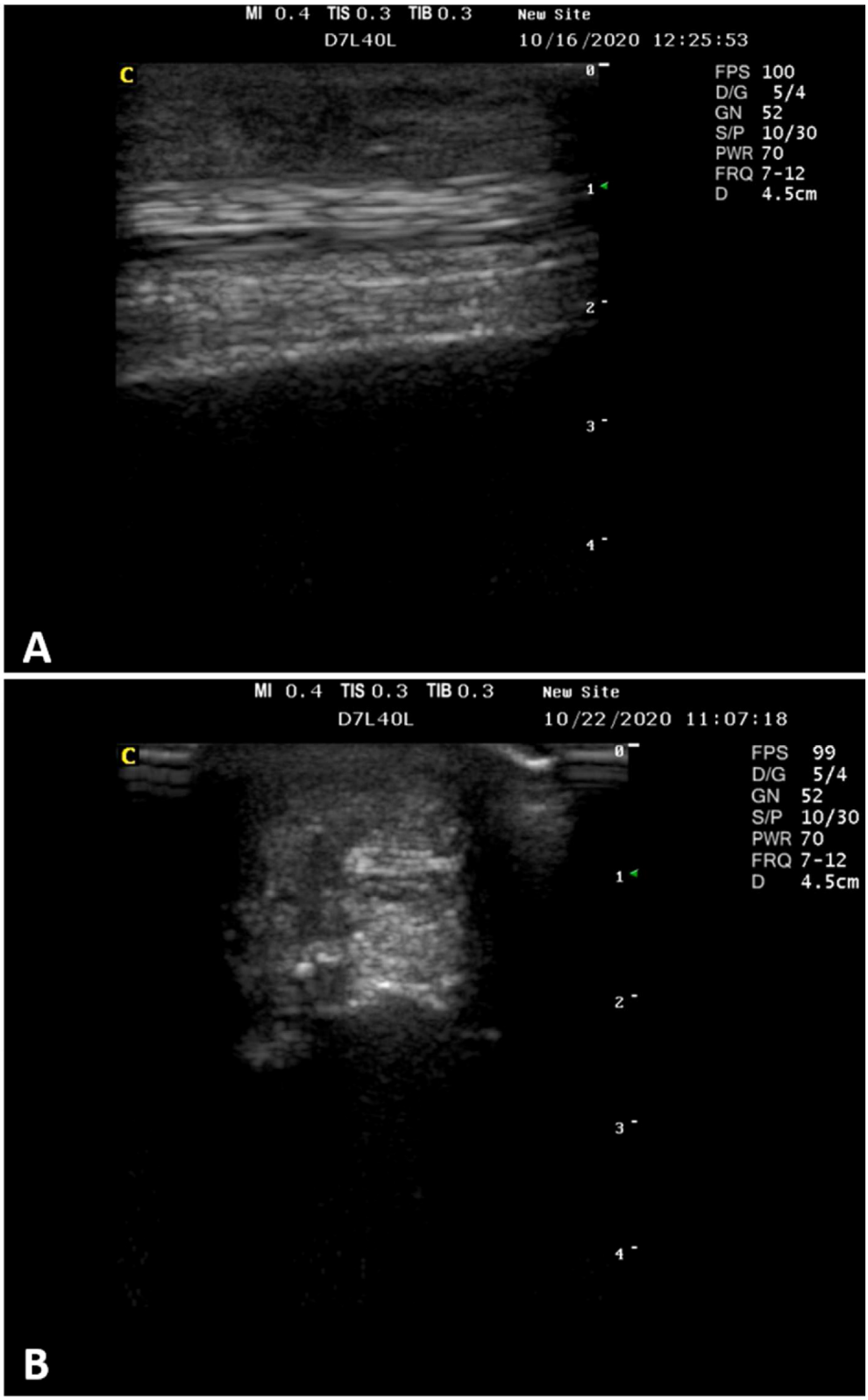

Fig. 10. Ultrasonographic appearance of superficial digital flexor tendon at 3 months (control group). A Longitudinal sonogram showing disoriented tendon fibers with subcutaneous reaction represented by anechoic fluids. The area of interest still thickened while the other segment retained to nearly normal thickness. B Transverse sonogram of superficial digital flexor tendon at three month postoperative showing irregular enlarged tendon.

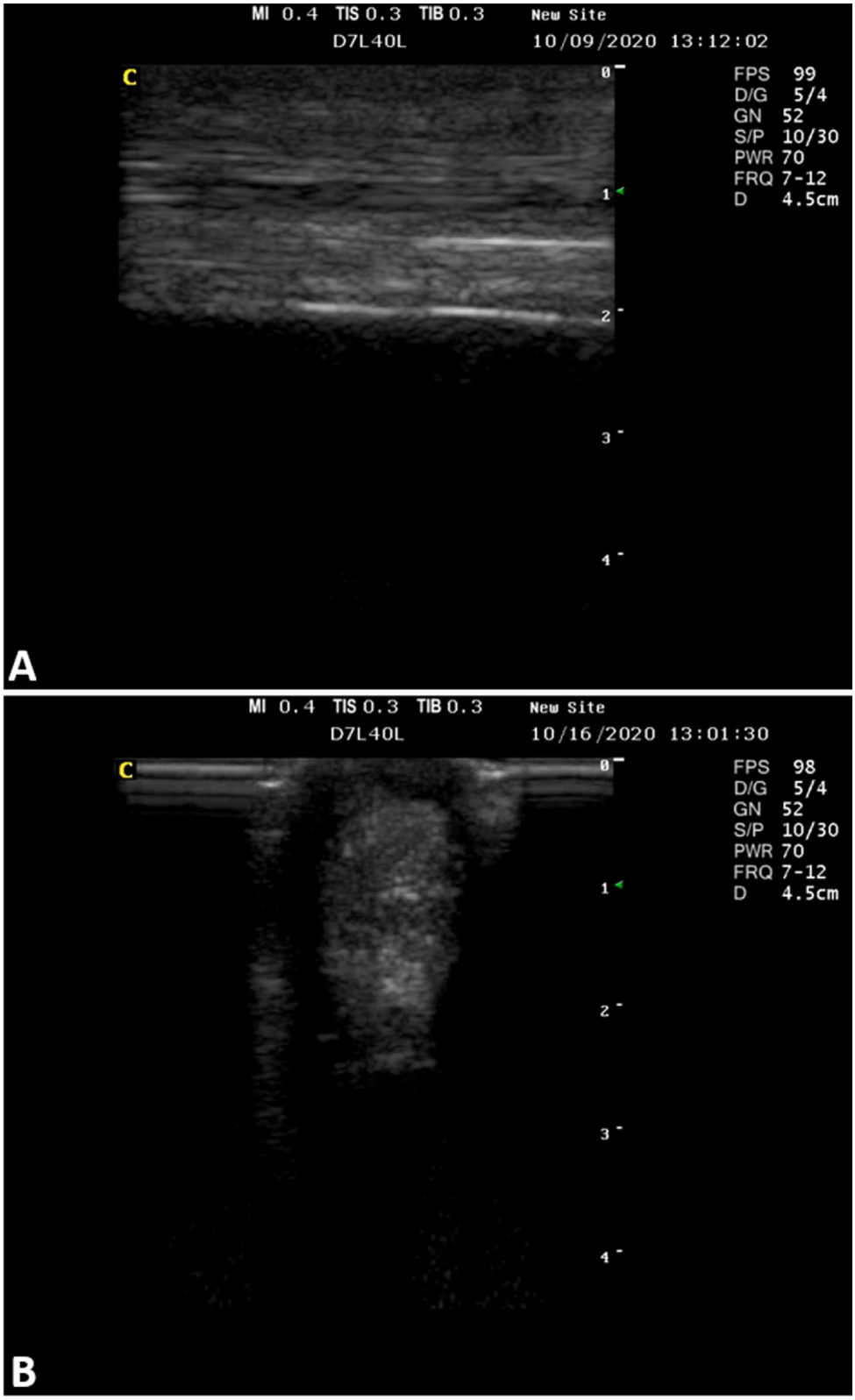

Fig. 11. Ultrasonographic appearance of superficial digital flexor tendon at three months (platelet-rich fibrin group). A Longitudinal sonogram Showing well-formed and oriented tendon fiber, close to total fiber alignment, with nearly normal thickness. B Transverse sonogram showing well-formed oriented displaced tendon with normal crescent shape.

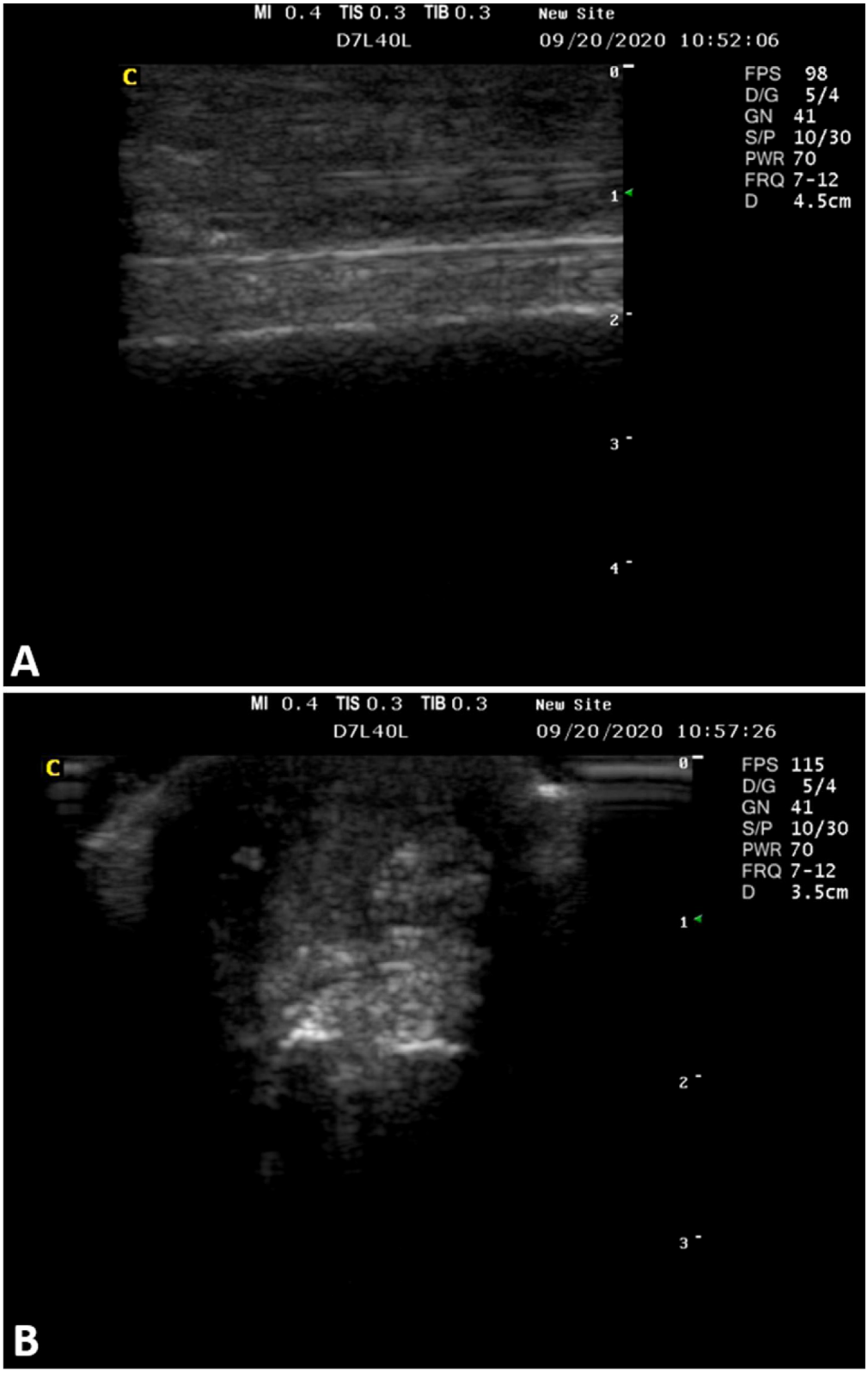

Fig. 12. Ultrasonographic appearance of superficial digital flexor tendon at three months (platelet-rich fibrin/chitosan group). A Longitudinal sonogram Showing well-formed and oriented tendon fiber, close to total fiber alignment, with nearly normal thickness. B Transverse sonogram showing well-formed oriented displaced enlarged tendon with normal crescent shape.

of the study, at 3 months, gradual increased echogenicity and decreased thickness were observed, and the fibers appeared more oriented and well-formed with minimal hypoechoic areas and anastomosis between the newly formed tissue and healthy tendon, which was attributed to the deposition of granulation tissue and scar tissue formation; however, there was no demarcation between the injured and normal tendon these results were also confirmed by Metineren [23]. They found that a progressive increase in echogenicity and fiber alignment reflects the associated collagen production, which increases the acoustic density. These results are attributed to the powerful regenerative role and anti-inflammatory effects of PRF via its growth factors [6].

The sonogram of the PRF/Ch group at 30 days showed the presence of complete anechoic content at the site of segmental defect with a marked increase in thickness with diffuse intratendinous edema in well-formed and well-oriented tendon fibers, while at 90 days, the tendon showed well-formed and oriented tendon fibers with minimal hypoechoic areas and a mild increase in thickness with a normal crescent shape, which can be attributed to the potential synergy in chitosan byproducts, when combined with growth factors, as supported by Rodríguez [24].

5. Conclusion

It was concluded that in the PRF and PRF/Ch groups, the fiber echogenicity, alignment, thickness, and shape position were closer to those of the native tendon, with a nonsignificant difference between them ($P > 0.05$). The results shown above indicate that PRF and chitosan accelerate and improve the quality of tendon healing as documented ultrasonographically and could provide a new bioscaffold-based strategy for tendon regeneration.

Animal ethics committee permission

the current research work was permitted to be executed according to the standards of the Animal Research Committee of the Faculty of Veterinary Medicine, Mansoura University, code No: M/21.

Author' contributions

M H: Study and experimental design, surgical procedures, statistical analysis, and research writing. A S: Study and experimental design, surgical procedures, research writing. A R: Study and experimental design, surgical procedures, revised manuscript. M E: Chitosan films preparation. G K: Study and experimental design, revised manuscript and supervising all studies.

Conflicts of interest

Conflict of interest statement: the authors declare that there is no conflict of interest.

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