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Comparison of clinical, biomechanical, and histopathological effects of various suture techniques on repair of tendon rupture by using autograft

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Abstract

Introduction: The purpose of this study was to compare the effectiveness of four different suture techniques in the treatment of experimentally modelled tendon injuries with tissue loss with autograft and grafting applications in rabbits. **Material and Methods:** The study was performed on 30 male mature (2-year-old) New Zealand rabbits with mean body weight of 3.1 kg, divided into three equal groups. A graft measuring 1 cm in length was collected from the *m. tibialis cranialis* of each rabbit under general anaesthesia. The graft collected from the right tendon was transplanted into the left tendon, and the graft from the left tendon was transplanted into the right tendon. In all groups, a simple interrupted suture was placed on the left tendon as control, a Bunnell-Mayer suture was placed on the right tendon in group I, a Locking-Loop suture in group II, and a Horizontal U suture in group III. Both hindlimbs were bandaged for four weeks. The tendons were assessed biomechanically and histopathologically. **Results:** According to the results of the tensile testing, the maximum durability of the techniques ranked as follows: Bunnell-Mayer, Horizontal U, Locking-Loop, and control groups. **Conclusion:** The use of autografts was a good alternative for the treatment of tendon ruptures with tissue loss. Furthermore, even though there were no clinical or histopathological differences, the suture technique can be chosen based on the results of the tensile test.

Keywords: rabbit, tendon repair, autograft, suture techniques.

Introduction

Tendon injuries are caused by various reasons and if they are not treated correctly, certain pathologies can occur, including increases in intramuscular connective tissue, decreased capillary density, early muscle fibre necrosis, insufficient muscle contraction, and irreversible contractures (5, 20, 23).

Cases of tendon rupture where apposition of the ruptured ends is not possible are treated with tendon lengthening surgeries, muscle/tendon transfer, or grafting applications (14, 15). Today, the most preferred suture techniques for these procedures are special tendon suture techniques such as Bunnell-Mayer, Krackow, Locking Loop, Three-Loop Pulley, Tsuge, Savage, Horizontal Mattress, and Mason-Allen. In tenorrhaphy, the suture material is reported to be as important as the preferred suture technique in terms of healing potential, and affects the biomechanics of the tendon (4, 8). Regardless of the preferred technique and suture material, many studies highlight that the affected extremity should be protected with a bandage for a certain time in the post-operative period (1, 4, 15).

The primary aim of the present study was to establish the feasibility of using a graft collected from a tendon on another tendon to provide a reconstruction model in cases of tendon injury with tissue loss where reconstruction was not possible. The secondary aim was to identify the most appropriate suture technique for grafting.

It is known that tendon healing is achieved due to intrinsic (10%) and extrinsic (90%) mechanisms (3, 8, 11, 13). Nevertheless, the working together of both mechanisms creates a synergic effect in order to bring about an ideal recovery (6, 8, 13, 15, 20).

In this study, the aim was to investigate healing or viability over time of graft material used when the ends of a tendon which has been ruptured could not be

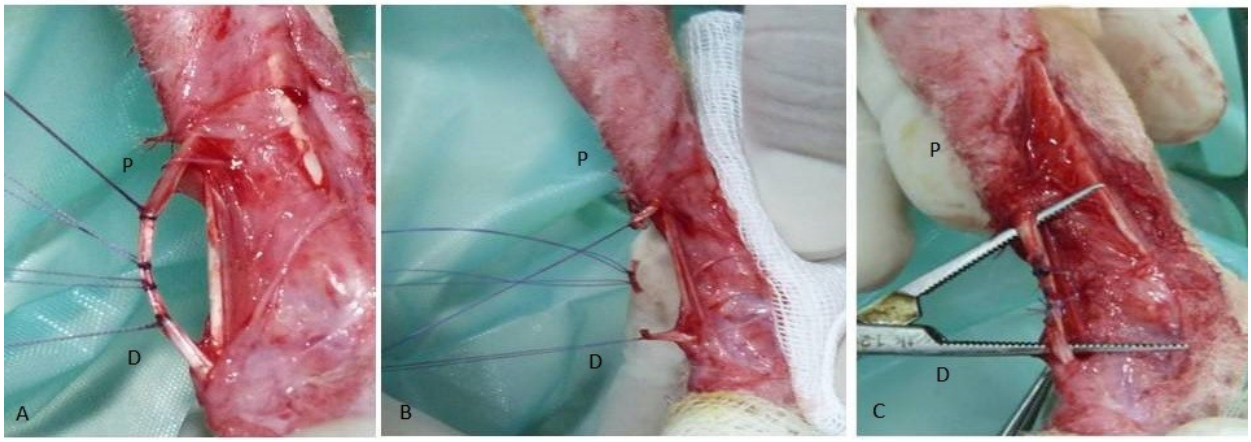

Fig. 1. Intraoperative view. A. Primary sutures being placed on the tendon and autograft; B. *M. tibialis cranialis* autograft and primary sutures; C. Appearance after transplantation

brought into contact or in the case of tendon injuries with tissue loss. Thus, the main theme of study was the hypothesis that tendon healing depends on extrinsic factors. Rabbits, which bear the larger part of their bodies on the hindlimbs, were preferred as the experimental model, and it was planned to apply the technique in the same or similar clinical cases (horse, cattle, dog, *etc*.) in case of the obtained results confirming our hypothesis.

Material and Methods

Animals. The study was performed on 30 mature (2-year-old) New Zealand white male rabbits with mean body weight 3.1 kg (range: 2.8–3.4 kg). Rabbits were purchased from the Experimental Animal Investigation Center of Ataturk University in Erzurum, Turkey. The animals were maintained in a temperature-controlled environment, illuminated for 12 h daily, and fed commercial pellets and water *ad libitum*. During the follow-up period, before and after the surgical procedure, the rabbits received feed and filtered water in separate containers and were maintained in individual cages. All procedures were carried out under aseptic conditions. The rabbits were divided into three equal groups. The right extremities of the rabbits constituted the material for group I (to receive Bunnell-Mayer sutures) (BM), group II (to receive Locking-Loop sutures) (LL), and group III (to receive Horizontal U sutures) (HU), and the left extremities constituted the material for the control group (to receive simple interrupted sutures) (SI).

Anaesthesia protocol. Anaesthesia was performed using a combination of 10 mg/kg/im of xylazine HCl (2% Rompun, Bayer, Germany) and 30 mg/kg/im of ketamine HCl (10% Ketasol, Richter Pharma, Australia). For three post-operative days, 50 mg/kg/im of metamizole sodium (500 mg/mL, Novalgin-Sanofi, Turkey) was used as an analgesic and 5 mg/kg/im of enrofloxacin for prophylaxis (5% Baytril, Bayer, USA), once daily.

Surgery. An incision measuring approximately 5 cm was made on the anterior of the tarsal joint of the right extremity, subcutaneous tissues were dissected, and the *m. tibialis* tendon was exposed. The tendon was completely separated from the surrounding tissue. To prevent the autograft from forming a crimp pattern during tenorrhaphy and to facilitate application, a fixation suture (United States Pharmacopeia size 4/0, polyglactin 910, synthetic, braided Vicryl, Ethicon, USA) was placed on both ends of the section of the tendon to be dissected as the autograft. Similarly, an additional suture was also placed 0.5 cm proximal to both sutures (Fig. 1A). The sutures, which were 0.5 cm from each other distally and proximally, were cut in the middle, and a graft measuring 1 cm in length was collected (Fig. 1B). The autograft was left among the tissue at the surgical site in order to help protect the viability of the autograft and prevent it from drying out.

The same procedures were repeated on the other extremity. The graft collected from the right side was attached to the left tendon using a SI suture. Then, the other autograft, which had been left among the tissues, was attached to the right *m. tibialis cranialis* using BM, HU, and LL suture techniques respectively in the relevant groups (Fig. 1C). The surgical site was closed using the routine method.

After the operation, a bandage with PVC splints was applied to both hind extremities with the tarsal joint in deep flexion. Bandage application continued for four weeks, and was changed every week.

At the end of six weeks, the rabbits were euthanised using a high dose of sodium pentobarbital (100 mg/kg). The tendons were assessed macroscopically for the presence of adhesion (Fig. 2) to the surrounding tissues and recorded as adhesion present (+) or adhesion absent (–).

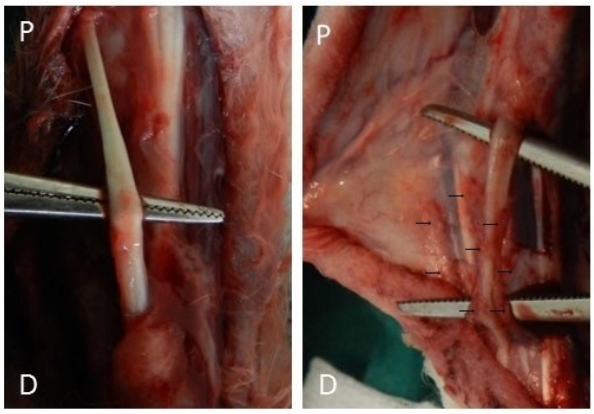

Fig. 2. The tendons with or without adhesion, groups II and III (Black arrows show adhesive tissues)

Each tendon was excised 2 cm from the proximal and distal anastomosis line where an autograft had been performed, including in the control group. A suture was then placed on the distal and proximal ends using silk thread in order to facilitate the placement of the tendons on the tensile testing machine, and the same sutures were used to surround the tendons (Fig. 3).

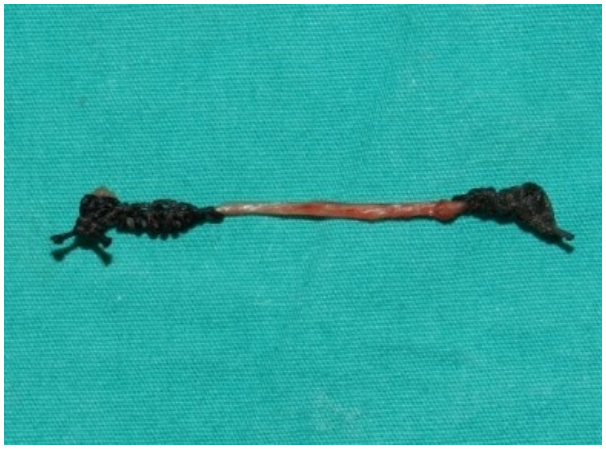

Fig. 3. Appearance of the autograft prepared for mechanical testing

The tendons were subjected to tensile testing on a screw-driven Shimadzu AG-IS mechanical stress machine (Shimadzu, Japan) with a load cell capacity of 100 Newtons. The results were recorded in Newtons (N) for statistical assessment (Fig. 4).

After tensile testing, the tendons were stabilised in 10% formalin. The samples were dehydrated in a TP 1020 Histokinette (Leica, Germany) with alcohol and xylene series and embedded in paraffin longitudinally. They were then cut into 5-micron sections using a RM2125RT microtome (Leica), and the preparations stained with haematoxylin and eosin (HE) were examined under an Eclipse E600 light microscope (Nikon, Japan).

Statistical analysis. Statistical analysis was performed using the Minitab-12 package programme (Minitab, USA). First of all, a normality test (Anderson–Darling Test) was conducted. Variance analysis was then performed on the continuous data (tensile strength) using one-way ANOVA and Tukey's test. Categorical data (the presence of adhesion) were assessed using the Kruskal–Wallis test, and suture techniques were compared with their controls by paired *t*-tests. All data were presented as mean ±standard deviation. P values <0.05 were considered statistically significant.

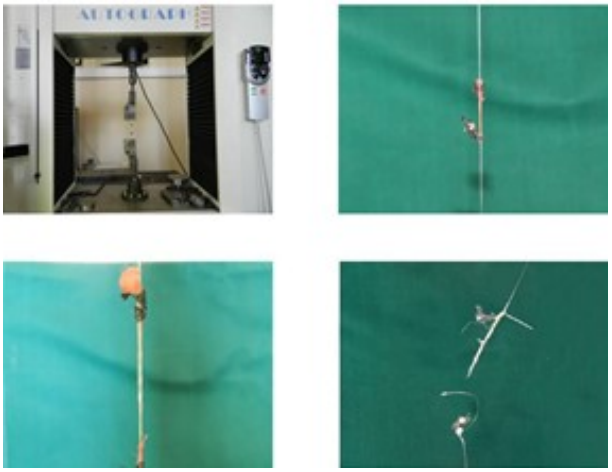

Fig. 4. Comparison of groups by using tensile machine

Results

Clinical observations. The rabbits were observed every day postoperatively to see if they could use their hind extremities. They stayed in a sitting position in their cages and did not walk for the first three days. Following the third day, they walked when stimulated, and roamed freely in their cages following the seventh day. There were no signs of infection in the wound area in controls. At the end of the fourth week there were no complications related to the operation areas when bandages were taken off and the animals were allowed to move freely within the cages.

Macroscopic examination. After six weeks, euthanasia was performed and the autografted tendons were isolated and assessed for the presence of adhesions (Table 1). While there was no statistically significant difference between group III and its control (P = 0.2039), statistically significant differences were found between group I and its control (P = 0.0087) and between group II and its control (P = 0.0318). No statistically significant difference was found among control groups (the control for group I, the control for group II, and the control for group III) or between the BM, LL, and HU groups (P > 0.05). Table 2 shows the median values for the groups, and Table 1 shows the presence of adhesions.

Biomechanical tests. The tensile tests showed that the tendons without adhesion ruptured at the anastomosis line, and the tendons with adhesion ruptured at a point other than the adhesion line. The data from the mechanical tensile tests show that the maximum tensile strength was 31.96 ±10.88 Newtons

(N) in group I, 16.62 ±7.38 N in the control for group I, 19.31 ±5.46 N in group II, 17.74 ±6.79 N in the control for group II, 23.13 ±7.32 N in group III, and 18.13 ±6.54 N in the control for group III. A statistically significant difference was found between group I and its control and between group I and group II ($P < 0.05$). Group I and group III were statistically similar ($P > 0.05$). No statistically significant difference was found between group II and its control or group III ($P > 0.05$). No statistically significant difference was found between group III and its control ($P > 0.05$). In Tables 3 and 4 the values of the groups and their statistical distribution are summarised.

Histopathological examination. No significant histopathological differences were found between the groups; the autograft was viable in all groups. The anastomosis line between the autograft and major tendon was filled with granulation tissue of fibrous character formed from the tendon sheath (endotendineum). These regions had mononuclear inflammatory cell infiltration characterised by occasional lymphocyte and macrophage infiltration and in rare instances contained suture material residue surrounded by foreign-body giant cells. Fibroblasts were very plump and shuttle-shaped, the organisation of the nuclei was not yet complete, and they moved in various directions. Furthermore, co-crimping, an indicator of elastin formation of collagen fibrils, was present. However, blood vessel and cell density was higher in this region than it was in normal tendons. In rare cases where fibroblast nuclei were present between the collagen fibres, fibroblast nuclei become thin and were located parallel to collagen fibres (Fig. 5).

Table 1. Adhesion in the groups

Presence of adhesions	Group I n = 10	Control for group I n = 10	Group II n = 10	Control for group II n = 10	Group III n = 10	Control for group III n = 10
+	7	1	8	3	6	3
–	3	9	2	7	4	7

Table 2. Statistical analysis of the presence of adhesions

Groups		n	Presence of adhesion (means ±SD)	P
Group I	BM	10	1.3 ±0.483	P = 0.005
	SI	10	1.9 ±0.316	
Group II	LL	10	1.2 ±0.422	P = 0.015
	SI	10	1.7 ±0.483	
Group III	HU	10	1.4 ±0.516	P = 0.081
	SI	10	1.7 ±0.483	

Table 3. Maximum force which the tendons were able to withstand before rupture (N = Newton)

No	Group I	Control for group I	Group II	Control for group II	Group III	Control for group III
1	28.6313 N	18.2469 N	11.4156 N	18.3000 N	30.6781 N	16.3000 N
2	38.3094 N	11.8375 N	19.6469 N	12.5000 N	29.8562 N	19.2000 N
3	40.7750 N	4.7218 N	27.7656 N	6.3000 N	14.6125 N	13.5000 N
4	49.4906 N	29.4469 N	19.2875 N	27.5000 N	16.2031 N	25.3000 N
5	33.1937 N	23.1938 N	12.1375 N	25.0000 N	15.0000 N	11.2000 N
6	11.4156 N	12.7643 N	15.3000 N	13.6000 N	26.2125 N	32.6000 N
7	32.2379 N	17.1534 N	23.8250 N	18.2000 N	20.2687 N	16.2000 N
8	34.4956 N	21.1472 N	20.0844 N	24.2000 N	35.4344 N	17.5000 N
9	33.1592 N	19.5376 N	25.8719 N	20.5000 N	18.3784 N	18.2000 N
10	17.8971 N	8.1862 N	17.7813 N	11.3200 N	24.6571 N	11.3000 N

Table 4. Median values of rupture point of tendons and statistical differences

Biomechanical assessment	Group I		Group II		Group III	
	BM	SI	LL	SI	HU	SI
Rupture point (Newton)	31.96 ^{a} ±10.88	16.62 ^{b} ±7.38	19.31 ^{b} ±5.46	17.74 ^{b} ±6.79	23.13 ^{ab} ±7.32	18.13 ^{b} ±0.54
$^{a, b}$ – different letters in the same row indicate statistical differences ($P < 0.05$)						

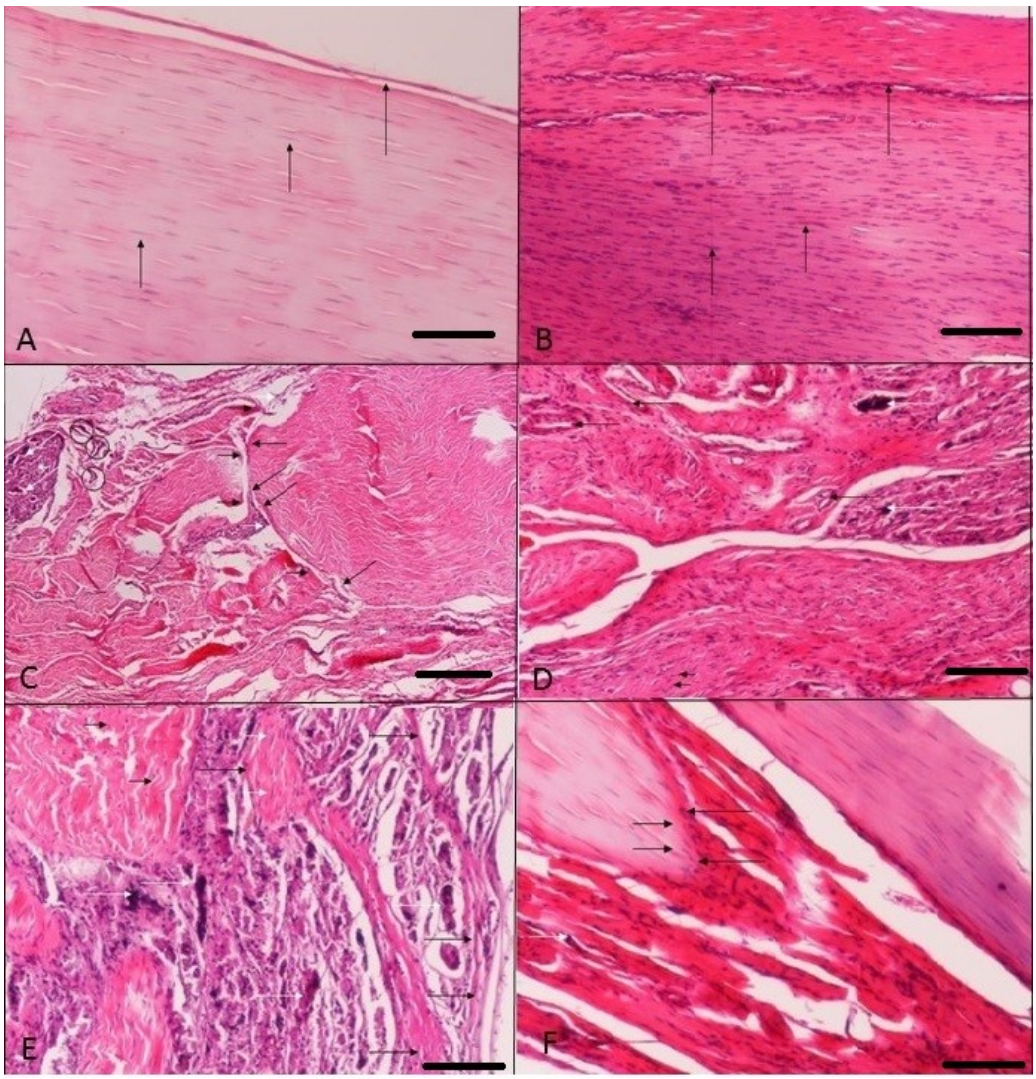

Fig. 5. Histological appearance of the cases. A. Longitudinal section of the anastomosis area of the host tendon. Smooth and parallel, thick collagen bundles. Fibrocytes with long and flat core (short arrows). Inner tendon sheath formed by the thickening of epitendineum (long arrow). HE, 100×; B. Loose connective tissue and capillary vessels located between the collagen bundles (endotendine) (long arrows). HE, 100×; C. Longitudinal section of a specimen from the HU group. Granulation tissue (short white arrows) with fibrous character originating from tendon sheath in the anastomosis area between autograft (short black arrows) and the host tendon (long black arrows). New shaped capillary vessels (black circles) with foreign body giant cells (long white arrow) and mononuclear inflammatory cell infiltration (white arrowhead). HE, 40×; D. Longitudinal section of a specimen from the LL group. New shaped arterioles and venules (long black arrows) with foreign body giant cells (white arrows). Parallel course fibrocytes (short black arrows) with thin core between some collagen fibres. HE, 100×; E. Longitudinal section of another specimen from the LL group. Collagen bundles (long black arrows) developing by the tendon sheath between fibrocytes (short white arrows) with long and flat core. Foreign body giant cells (long white arrows) in granulation tissue between collagen bundles. HE, 100×

Discussion

Tendon lengthening surgeries are performed for the treatment of tendon injuries with tissue loss or those where the apposition of ruptured ends is not possible because of elapsed time. However, there are disadvantages such as thinning of the tendon in the section that is lengthened and decreased suture reliability. Therefore, recent studies have been directed to procedures such as tendon transplantation, autograft, homograft, and heterograft (11, 14, 19, 22). However, it is known that biomechanical and histopathological studies where free flap autograft is performed and which demonstrate the role of suture techniques in tendon healing are not sufficient (21, 27). The present study aimed to investigate the effectiveness of free autograft use to eliminate the disadvantages reported with the preferred tendon lengthening methods in reconstructing tendon ruptures with tissue loss. For this purpose, a large experimental defect was induced in *m. tibialis cranialis* in rabbits, and reconstruction was achieved through mutual transplantation of the autograft collected from the same tendon in the other extremity.

There are reports indicating that caution should be undertaken in the reconstruction of vessel-poor tissues

such as tendons and ligaments (19). Therefore, careful selection of the surgical instruments to be used in tendon fixation is as important as the suture material used in tendon operations. In the present study, four sutures were placed prior to removing the graft taken from the tendon to prevent damaging the graft ends during fixation. It is safe to say that the fixation sutures provided significant convenience when attaching the graft to the major tendon without damaging it and causing a crimp pattern during transplantation.

A multifaceted assessment suggests that the BM, Krackow, LL, and Horizontal Mattress suture techniques are more frequently preferred in tendon surgeries (1, 3, 4, 7, 23). Pijanowski *et al.* (25) compared the tensile (resistance) strength (durability) of four different suture techniques and found that the SI and Mason-Allen suture techniques had very similar but lower rupture strengths compared to the BM and Kessler suture techniques. The results of the biomechanical assessment conducted in this study showed that the durability ranking of the tendons from different groups was as follows: BM 1, HU 2, LL 3, and SI 4. The BM suture technique, which is preferred particularly in the reconstruction of round and almost round extensor tendons because it does not affect the circulation of the tendon to a high degree and prevents gap formation between the tendon ends, is frequently used in large animal surgeries as well (8, 9, 12, 24). The results obtained from the study suggested that the rupture strength of the BM suture technique is higher than that of the other groups. In this regard, the results of our study were consistent with the data from the literature.

Studies report that the LL suture technique effect on the supply to tendons is relatively less severe, and due to its locking mechanism, it reportedly prevents the strand from pulling out from the tendon (10, 12, 16, 17, 26). Histopathological examinations showed that although the LL technique provides sufficient support for reconstruction between tendon ends, no statistically significant difference was found when compared to the other groups. However, in view of the data obtained from the study, the LL suture technique had lower mechanical resistance (19.33 ±5.44 N) than the BM and HU techniques. Due to its locking mechanism, the initial mechanical resistance in the LL suture technique is reportedly high (2, 16–18). Nevertheless, the resistance measured at the end of six weeks was lower than the BM and HU techniques. The possible cause of it was the negative impact of the locking mechanism on supply to the tendons.

Following tenorrhaphy, inflammatory reactions develop in surrounding tissues. Healing tissue formed as a result of the migration of fibroblasts from the surrounding soft tissues to the tendon ends during the healing process of the tendon causes adhesion (1, 4, 8). In our study, no measure was taken to prevent adhesion. When the presence of adhesion was assessed in the experimental groups, we saw that adhesion developed between the tendon on which tenorrhaphy was performed and the surrounding tissues in seven subjects in group I (BM), eight subjects in group II (LL), and six subjects in group III (HU). While scar formation at the anastomosis line was thought to have a positive impact on mechanical resistance, the durability in group II was lower than in groups I and III although the adhesion was higher. In the control groups where the SI suture technique was used, both the formation of adhesion and the rupture strength was lower than in other groups. The fact that rupture strength was lowest in group II, which had the highest rate of adhesion, and in the control groups, which had the lowest rate of adhesion, indicates that adhesion has no effect on mechanical resistance.

Aygün *et al.* (4) have reported finding granulation tissue comprised of vessels and cells between the healing tendon ends and determined that fibrocytes developed in parallel with newly formed collagen fibrins similar to normal tendon healing. In our study, it has been observed that autograft was viable histopathologically. The autograft and host tendon anastomosis lines were found to be filled with fibrous granulation tissue developed from the tendon sheath (endotendineum). In this region, mononuclear inflammatory cell infiltration was detected, characterised by occasional lymphocyte and macrophage infiltration. It was found that fibroblast nuclei, which are rarely found among the developing collagen fibres in these regions, began to be thinned and appeared to be parallel to the collagen fibres. In view of the histopathological data, the findings of our study and histopathological findings of previous studies (1, 4, 8) are consistent to a large extent.

In conclusion, when the clinical, biomechanical, and histopathological data were evaluated together, autograft could be a good alternative for the treatment of tendon ruptures with tissue loss, and any of BM, LL, or HU suture techniques may be selected to fix the autograft. As an experimental model this study gave positive results in the repair of tendon ruptures. Therefore, it is concluded that the same model is also worth trying in clinical cases with tissue-loss tendon injury or tendon shortness.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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