Role of autologous platelets rich plasma in treatment of experimentally induced Achilles tendonitis in rabbits

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Abstract
The study aimed to a clinico-histopathological evaluating the benefit of using autologous platelets rich plasma (PRP) in healing of Achilles tendonitis in rabbits. Twenty adult male rabbits were used. Five ml of blood was withdrawn from the rabbits marginal ear vein and mixed with sodium citrate for preparation of PRP. Rabbits were randomly allocated into two groups (10 of each). The first group, serve as a control group and the second group, considered as treatment group. Tendonitis was induce under the effect of general anesthesia. Lateral longitudinal incision on the skin over the Achilles tendon was made. The tendon was isolated by blunt dissection from the surrounding tissue. Tendonitis was induced by splitting of the tendon with surgical blade. The first group (treated with one ml normal saline). In contrast, the second group (treated with one ml of PRP). Both saline and PRP were injected intra-lesional after that the surgical skin wounds was re stitched in routine manner. After clinical follow-up of the treatment rabbits, certain secondary complications were happened represented by lameness, swelling and infection. Histo-pathological evaluation was performed at 8 and 16 weeks post-surgery (10 rabbits/group) (5 rabbits/period). Grossly, adhesion was noticed in most rabbits of control group. Microscopical examination reflect perfect orientation and organization of collagen fibers in treatment group in comparing with control group. Based on the results obtain from this study, PRP enhanced and promote tendon healing.

Key words: PRP, Achilles tendonitis, rabbits

دور البلازما الغنية بالصفحات الذمىية الذاتية في علاج التهاب وتز أكيلس المستحث تجريبيا في الارانب

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الخلاصة
هدفت الدراسه الحالية الى التقييم السريري و المرضى-النسجى لاستعمال البلازما الغنية بالصفحات الدموع الذاتية في شفاء وتز أكيلس بعد تخطيه في الارانب. استخدمنا لبحث عشرون ارنبًا بالغًا. سحب 5 مل م من الوريد الارناني ومزج مع الصوديوم ستريت لتحضير البلازما الغنية بالصفحات الدموع. قسمت الارانب الى مجموعتين يوافق (10 ارنب لكل مجموعة). عدت المجموعة الأولى مجموعة سيطرة وثانية مجموعة المعالجة. تم احداث التهاب الوتر تحت تأثير التخدير العام ثم شق الجلد المغطي للوتر طولاً من الجهة الوحشية وارتجج طية الجلد وعزل الوتر من الأنسجة المحيطة به. أحدث التهاب الوتر في الارانب بالأسفلة التشريطي بالстроен الجراحي. عولجت المجموعة الأولى بالصورلي 1 مليلتر من المحلول الفصلي. أما المجموعة الثانية فاستعمل لها 1 مليلتر من البلازما الغنية بالصفحات الدموع. حققت المادتين داخل الوتر ثم اغلق الجلد بالطريقة الروتينية. تبعت البحوث صربيا حيث سجلت بعض المضاعفات الثانوية شملت على العرج والتورم والخمج. أجري الفحص المرضي-النسعجي بعد 8 و16 أسبوع من إجراء المداخلات الجراحية حيث استخدمت 10 أرنب لكل مجموع (يوافق 5 أرنب لكل فترة). لوحظ عيانا التصاصات بين الوتر والانسجة المحيطة به في أغلب البحوث السيطرة. أظهر الفحص المجري تغييرات انظام الجذور الكولاجين في مجموعة العلاج مقارنة بنوعية السيريط. استنادا الى النتائج المستخلصة من الدراسه لم تنح البلازما الغنية بالصفحات الدموع عجلت في شفاء الوتر.

الكلمات المفتاحي: - البلازما الغنية بالصفحات الدموع - التهاب وتز أكيلس - ارنب
Introduction

In man and animals the etiology of Achilles tendon injuries is usually traumatic. The nature of the trauma influences the type of tendon injury and may result in acute rupture of the tendon with partial or complete loss of integrity of the structure (1). In addition, weakening or rupture of the tendon structure can occur secondarily to systemic diseases (e.g. Cushing’s disease) or to iatrogenic etiologies (2). The clinical signs associated with the rupture of the Achilles tendon will vary depending on the severity of the injury, but the swelling around the tendon insertion on the hock is the main symptom (3). Tendon ruptures are initially treated by direct suturing techniques as a standard of care and tendon transplantation and stem cells (4). Postoperative adhesions are still encountered. For these reasons, treatment of tendon injuries, is a state of art and depends on the surgeon’s experience, equipment, facilitation, and condition (5). A repaired tendon needs to be protected for weeks until it gains enough strength to handle physiological loads (6). Recently platelets also release substances that promote tissue repair and influence the reactivity of vascular and other blood cells in angiogenesis and inflammation is used which contain storage pools of growth factors(7, 8 and 9). Thus the present study is assigned to evaluate the benefits of using autologous PRP in healing of Achilles tendonitis following its destruction by splitting in a rabbit model.

Materials and methods

1. Experimental animals:
Twenty adult male rabbits of 10 ± 2 months age and 1.700 ± 0.150 kg body weight were enrolled for this clinical prospective study. They were placed in standard rabbit cages, under the same conditions. Rabbits were randomly allocated into two groups (ten of each). The first group serve as a control group and the second group considered as treatment group.

2. Preparation of platelets rich plasma (PRP):
PRP was prepared as described by (10) as follow: The rabbits were sedated with xylazine 2% injected IM. Under a septic technique five ml of blood was withdrawn from the rabbits marginal ear vein and mixed with 3.2% sodium citrate as anticoagulant at a ratio of one ml sodium citrate to five ml of whole blood. The sterile test tubes containing blood were centrifuged at 3000 rpm for 15 minutes to separate the platelets poor plasma (PPP) from red blood cells and puffy coat. After the first centrifugation, the blood was separated into plasma and red blood cells. The plasma was aspirated pipets and put in sterile test tube and the red blood cells were discarded and after a further centrifugation of the remaining plasma for five minutes. The bottom layer, which was rich in platelets was collected for use as PRP with a concentration of 400 to 560×10^3/μL at the time of using. The PRP was stored at 4 C° until it was injected into the wounded areas of tendon.

3. Tendon injury model and local injection of PRP:
Rabbits were fasted for 12 hours prior to the operation, then anesthetized using a combination of ketamine hydrochloride 5% (Claris, India) at a dose rate of (35) mg/kg and xylazine hydrochloride 2% (Rompun® - Bayer) at a dose rate of (10) mg/kg injected intramuscular (11). The site was prepared for aseptic surgery (fig. 1). Lateral longitudinal incision on the skin over the Achilles tendon was made , a skin flap was reflected. The tendon was isolated by blunt dissection from the surrounding tissue with a small curved forceps. Simulation of a rabbit’s Achilles tendon rupture was made by splitting with surgical blade midway between its calcaneal
Exposure of Achilles tendon and splitting is done with surgical blade.

Injection of PRP into the injured tendon.

insertion and the musculo-tendinous junction (Fig. 2). The first group (treated with one ml normal saline 0.09%). In contrast, the second group (treated with one ml of PRP) (fig. 3). Both saline and PRP were injected intralesion after that the surgical skin wounds were reconstructed by simple interrupted pattern with polypropylene suture USP 4-0.

4. Histopathological evaluations:
Tendon Biopsies were collected from (20) rabbits (10 rabbits / group) and (5 rabbits / period) in 8 and 16 weeks post-treatment. Biopsies were fixed in 10% neutral buffered formalin, and then dehydrated through ascending grades of ethanol. Cleaning was done with xylene. The processed tissue was impregnated in paraffin wax inside oven at 60 C° overnight, then sectioned by a microtone at 5-6 μ thick and mounted on glass slides and stained with Hematoxyline-Eosin stain (H & E). The slides were examined under light microscope equipped with photo-automatic unit (12).

Results
1.Clinical follow-up:
All animals used in this study well tolerated the surgery. Detectable lameness persisted in all animals directly following recovery from anesthesia. Lameness was severe in control group and lasted for the first week post-surgery. In treatment group lameness was mild to moderate and disappeared in 3-5 days post-surgery. And after that time the weight bearing of affected limb was same as in contralateral limb (no lameness). Signs of infection or adverse outcomes of surgery were seen in two rabbits related to control group and one related to treatment group in 9th and 12th days post-surgery respectively. The infection was superficial caused dehiscence of some skin stitches. Also local swelling at the operative site was seen in some rabbits which lasted for few days. Swelling was moderate in control group and mild in treatment groups and remitted faster in the later. No any treatment was used and swelling was diminished spontaneously.

2. Macroscopical examination:
The tendons injected with saline were thicker than the tendons injected with PRP. Most control tendons had adhesions (6 rabbits out of 10) when comparing with PRP treated tendons (2 rabbits out of 10) and were adherent to the surrounding tissue and the superficial fascia (figure 4).
There was no gliding when the foot was moved, and their incised ends were bulbous. The PRP treated tendons were easily separated from the skin, clearly delineated, and moved easily when the foot was being moved. Their incised ends were distinct and smooth. PRP treated tendons showed less peritendinous fibrous tissue than the control tendons.

3. Microscopical examination:
The pathognomonic features of microscopical examination in both groups are illustrated in table (1).

<table>
<thead>
<tr>
<th>Time (wks)</th>
<th>Control group</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Presence of inflammatory cells and perivascular edema (fig.5).</td>
<td>Regular collagen fibers and presence of few capillaries (fig.6).</td>
</tr>
<tr>
<td>16</td>
<td>Regular organization of collagen fibers and moderate increase in fibroblasts (fig.7).</td>
<td>Regular orientation of collagen fibers and tenocytes which have cigar shaped nuclei are arranged along the longitudinal axis of the tendon (fig.8).</td>
</tr>
</tbody>
</table>

Fig.(5) Tendon of control group, 8 weeks post-surgery show inflammatory cells (thick arrow) and perivascular edema (empty arrow) (H&E., X100).

Fig.(6) Tendon of treatment group, 8 weeks post-surgery show regular collagen fibers (thick arrow) and presence of few capillaries (empty arrow) (H&E., X100).

Fig.(7) Tendon of control group, 16 weeks post-surgery show regular organization of collagen fibers (thick arrow) and moderate increase in fibroblasts (empty arrow) (H & E, X100).

Fig.(8) Tendon of treatment group, 16 weeks post-surgery show regular orientation of collagen fibers (thick arrows) and tenocytes which have cigar shaped nuclei (empty arrows) are arranged along the longitudinal axis of the tendon (H&E, X100).
Discussion

1- Clinical observations:
In the present study, some minor non-specific secondary health problems were encountered which include (lameness, swelling and infection). Lameness may be associated with pain evacuated from surgery. These findings are in line with earlier reports by (13) whom observed lameness in rabbits due to pressure resulted from accumulation of blood and inflammatory exudates associated with a high concentration of chemical irritants, like lactic acid may be responsible for generation of pain in injured tendon, which then lead to appearance of lameness. In the present study the ability of the animals in the treatment group to use the operated leg is better than the animals in the control group in which the animal exert great efforts in order to stand and tend to remain recumbent most of the time and standing occurred after several trials as compared to those of the treatment group. The gait of the animals of the both group was observed clinically two weeks after operation. The treatment with PRP was considered successful when the animals return to the same pre-injury movement without relapse of the injury in the follow-time. While the control animals suffered from lameness for longer period when compared with treatment animals. These signs also noticed by (14). Local swelling that happened may be attributed to the formation of intratendinous edema and influx of inflammatory cells, as a part of the inflammatory process. In a study in rat (15), recorded swelling in all animals which disappeared in a period of (2-3) weeks. Finally infection which took-place in three rabbits may be ascribe to bacterial infection and this a common complication following surgery. This complication was treated by debridement of skin edges then washed with normal saline 0.9% and finally with povidon iodine and restitched of the skin in addition to administration of systemic antibiotics for five consecutive days and all wounds healed primarily but relatively with a longer time comparing with the remaining rabbits. Infection was noticed in previous studies (16) in rats and (17) in rabbits. In the present study the secondary complications were diminished in a short period in PRP treated group when compared with saline group.

2. Macroscopical examination:
The main gross finding is the peritendinous adhesion which were happened in most animals of control group and consider a major problem in hand surgery. Adhesion may be happened due to scar tissue formation, which disrupts tendon gliding. Peritendinous adhesion were noticed in previous studies (18 and 19). Furthermore adhesion as mentioned by (20) has got great limitation in movement and the tendon can no longer glide freely and may impair the function of limbs seriously. Adhesions are part of the healing process and almost inevitably produce functional disability following the biological response of the tendon to injury. Post-operative mobilization decreases adhesion formation and improves function and repair of Achilles tendon (21).

3. Microscopical examination:
Microscopical examination of biopsies obtain from both groups show perfect and faster tendon healing in PRP group in comparing with control group. These findings are in accordance with (22), who showed that the injection of PRP within the Achilles tendon could increase cellular density and promote neo-vascularization. On the other hand, the present study showed that the angiogenesis decreased in the PRP group significantly at the end of experiment in comparison with the control group. Thus, the decrease of vessel density in combination with the orientation of the collagen fibers and presence of tenocytes indicates that PRP accelerates the whole healing process. Other researchers (23, 24 and 25), indicated that the activation of the platelets of PRP leads to release of conspicuous amounts of various growth factors including platelet-derived growth factor (PDGF), transforming growth factor (TGF), platelet derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-I, platelet-factor (PF-4) and epidermal growth factor (EGF). This may be due to the effect of PRP. In rabbits, PRP injection increased ultimate strength and stiffness when applied to repair of the patellar tendon because of the presence of an increased concentration of
growth factors (26). In conclusion the healing of tendon seem to be a complicated process and the use of PRP had a positive effect on functional improvement of injured tendon and enhanced healing in comparing with control group.

References