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Expression of transformating growth factor beta 1 (TGF- β1) in the healing process of a mouse achilles tendon rupture (Rattus norvegicus) given platelet rich plasma

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ABSTRACT

Introduction: Platelet rich plasma (PRP) contains not only several growth factors but also proinflammatory cytokines that promote the healing process of tendon ruptures. Therefore, this study aimed to determine the effect of PRP administration on TGF- β1 expression during the healing process of Achilles tendon rupture in white rats (Rattus norvegicus).

Method: This study was an experimental research with a Posttest-Only Control Group Design approach. This study enrolled 30 male white rats with a ruptured Achilles tendon, aged 3-4 months and weighing 200-250 grams. The rats were randomly divided into 2 groups. The first (I) group as the control group included rats with Achilles tendon rupture without PRP administration, and group II as the experimental group included rats with Achilles tendon rupture with PRP 100 ul administration. Equal number of white rats from both groups were observed in 3 different times (on the day 7th, 14th, and 21st) after intervention. Part of the substance of the right Achilles tendon of each white rat was taken for examination of TGF-β1 expression by immunohistochemical examination with anti-TGF- β1 primary antibody. The data were analyzed by using SPSS 25.0. The descriptive data were provided in table, and the analytic data were analyzed by using unpaired t-test. The significant p value was <0.05.

Results: We established that the intensity of TGF- β1 expression of Achilles tendon in the experimental group (with PRP administration) was statistically significant (P<0.05) compared to the control group. TGF- β1 expression in the experimental group established stronger and more extensive staining at weeks 1 and 2 (P < 0.05), while the control group established more extensive staining at week 3 (P < 0,05).

Conclusion: PRP administration can increase TGF-β1 expression during the healing process of Achilles tendon rupture in white rats (*Rattus norvegicus*) and its statistically significant.

Keywords: platelet-rich plasma, TGF-β1 expression, achilles tendon rupture, rats.
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INTRODUCTION

Achilles Tendon (AT) is the strongest and thickest tendon in the human body. Achilles tendon serves to attach two muscles, gastrocnemius and soleus.[1] Achilles tendon gets through three joints, which are knee joint, ankle joint and subtalar joint. Function of Achilles tendon are for walking, running and jumping.[2] Even though achilles tendon could resist high pressure when running and jumping, however it is still susceptible to rupture and injury.[3,4]

Achilles tendon rupture (partial or complete), is a rupture or separation of the tendon fibers thus the Achilles tendon can no longer perform its function.[5] Achilles tendon rupture is caused by trauma or due to overuse of the Achilles tendon.[6] Incidence of Achilles tendon rupture is the most common in developed countries with different prevalence. The epidemiology in North of America, reported the incidences from 5.5 to 9.9 cases per 100,000 people, however in Europe it ranges from 6-37 cases per 100,000 people.[7–9] Achilles tendon rupture is commonly found in adult men who actively participate in sports activities (44 - 83%).[8] Achilles tendon ruptures are more common on the left side than on the right side of foot, because most people are right-sided and depend on left foot.[9]

Concern for patients with achilles tendon rupture is healing time that is quite long, then it will disrupt the patient's activities and productivity in daily living. Patients with an Achilles tendon rupture, 45% of them will experience decreased of mobility and 25%-75% of them will be unable to walk independently or achieve a level of independent living within 1 year of injury.[10]

Activation of Transforming Growth Factor Beta 1 (TGF-β1) increases the healing process of tendon ruptures. It is one of the TGF- β1 superfamily polypeptides produced by platelets, osteoblasts, bone marrow stromal cells (BMSC), chondrocytes, endothelial cells, fibroblasts and macrophages.[11] TGF1-β had a pivotal role as a broad biological regulation processes of tendon ruptures.[12]

Platelet rich plasma (PRP) is part of the autologous blood plasma fraction which has a 3-5 times platelet concentration above the normal value (>450,000/mm^3).[13,14] Platelets play an important role in haemostasis, tissue regeneration, new connective tissue construction, and revascularization.[15] PRP not only rich of thrombocytes, but also contains coagulation factors, cytokines and growth factors such as Platelet Derived Growth Factor (PDGF), Transforming Growth Factor Beta (TGF-ß1), Insulin like Growth Factor (IGF), Fibroblast Growth Factor (FGF), Platelet Derived Angiogenesis Factor (PDAF), Platelet Derived Endothelial Growth Factor (PDEGF), and also Vascular Endothelial Growth Factor (VEGF) which are important for the healing process of tendon injuries.[13,14,16] The growth factors contained in PRP serve to accelerate endothelial, epithelial and epidermal regeneration, as an angiogenesis stimulation, synthesis of collagen, enhanced the healing process of soft tissue. It also balancing the homeostasis and reduce the scar of the tissue.[17,18]

METHODS

This research is an experimental research with a Posttest-Only Control Group Design approach on white rats (*Rattus norvegicus*) as experimental animals. This research divided into two groups of sample, group I as a control group include white rats with right achilles tendon rupture which were sutured primarily with modified Kessler suture using prolene 5.0 and strengthened with running suture using prolene 6.0 without PRP administration. Group II as an experimental group include white rats with right Achilles tendon rupture that received intervention. These group II white rats were sutured primarily with modified Kessler sutures using prolene 5.0 and strengthened with running sutures using prolene 6.0 then received PRP administration. The healing process of each group with achilles tendon rupture was observed 3 times, the first observation was in the 7th day after intervention, the following observation was in the 14th day and 21st day after intervention. After that, to measure the TGF-β1, part of the substance of the right Achilles tendon of each white rat was taken for examination of TGF-β1 expression by immunohistochemical examination with anti-TGF-β1 primary antibody. The data were analyzed by using SPSS 25.0. The descriptive data were provided in table, and the analytic data were analyzed by using unpaired t-test. The significant p value was <0.05.

RESULTS

We reported the distribution of TGF-β1 expression of achilles tendon tissue in the control group (P0) and the experimental group (P1) continued to change after intervention by the time of observation (days 7th, 14th and 21st). After the 7th day of observation, distribution of TGF-β1 expression in tendon tissue of P1 was higher than P0. On day 14, the conveyance of TGF-β1 expression expanded in both groups, with a higher level of conveyance within the test group than the control group. In the 21st days after intercession, the distribution of TGF-β1 expression within the P1 was diminished, whereas expanding within the control groups

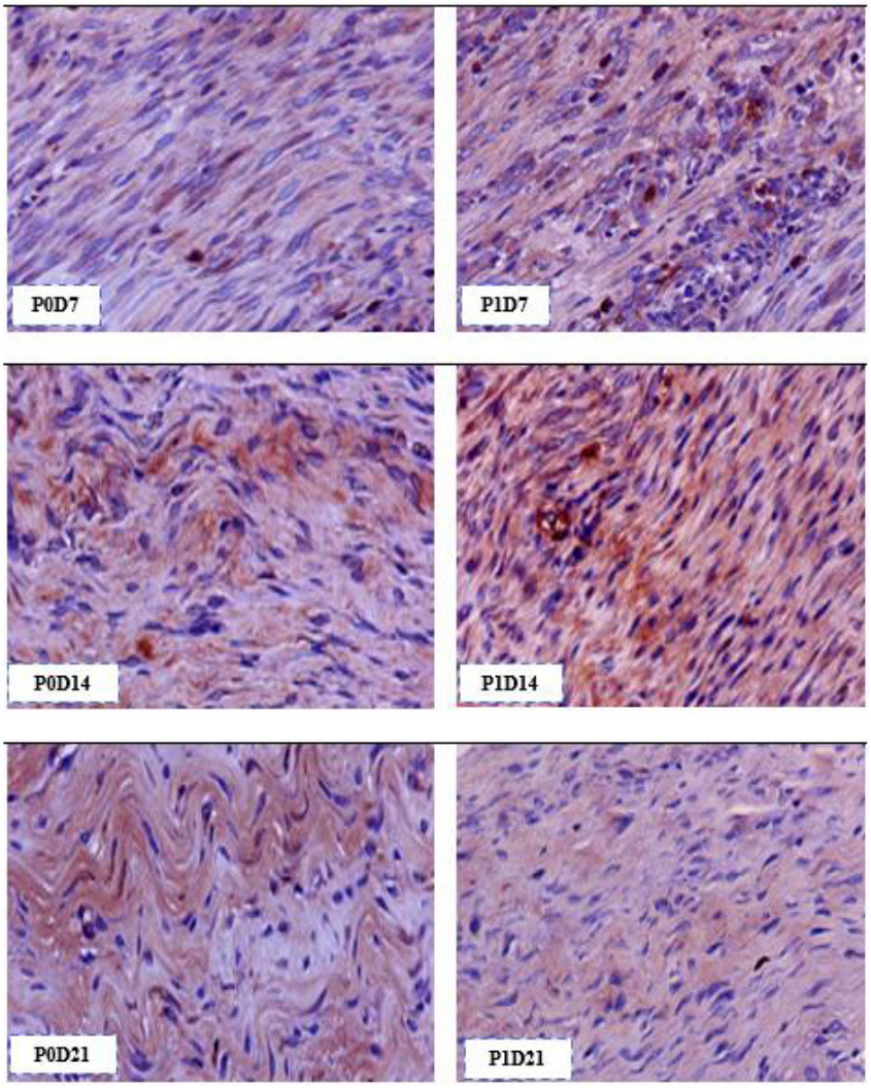

Figure 1. Photomicrograph of (TGF-β) expression in the Achilles tendon tissue of white rats (*Rattus norvegicus*) in the control group (P0) and experimental group (P1) observed on the 7th, 14th and 21st days (D) after rupture with immunohistochemical staining on 400x magnification.

and higher dissemination than the test groups. The distribution of TGF-β1 expression in P0 and P1groups served in the photomicroscopy observed on the 7th, 14th and 21st days after tendon rupture intervention and PRP administration. The photomicroscopy results using immunohistochemical staining examination of 400 times magnification can be seen in Figure 1.

The level of TGF- β1 expression as a result from immunohistochemical staining of the achilles tendon tissue in this research was assessed by intensity score which was described as low intensity (score 1), moderate intensity (score 2), and high intensity (score 3). The average intensity score of TGF - β1 expression in the Achilles tendon tissue of white rats (*Rattus norvegicus*) in the control group (P0) and the experimental group (P1) observed on the 7th, 14th and 21st days after tendon rupture can be seen in Table 1.

From Table 1 above, it can be seen that the average intensity score of TGF- β1 expression in the control group increased on the 7th, 14th and 21st days, while the experimental group increased from day 7th and peaked on the 14th day, then decreased on the 21st day. The average intensity of the TGF - β1 expression score in the tendon tissue of the control group on the 7th, 14th and 21st days after intervention, respectively, was 1.40 ± 0.55. ; 1.80 ± 0.45 and 2.40 ± 0.55 while the experimental group of rats were 2.20 ± 0.45, respectively; 2.80 ± 0.45 and 1.80 ± 0.45. The histogram of the average IS expression of TGF - β1 in the achilles tendon tissue of white rats in the control and experimental groups observed on the 7th, 14th and 21st days after intervention can be seen in Figure 2.

Table 1. The mean (± SD) intensity score of TGF - β1 expression in the Achilles tendon tissue of the *Rattus norvegicus* in the P0 and P1 groups were observed on the 7th, 14th and 21st days after tendon rupture intervention.

Intervention	Time of Observation			Mean
	D-7	D-14	D-21	
P0	1,40 ± 0,55 ^{aA}	1,80 ± 0,45 ^{bA}	2,40 ± 0,55 ^{bA}	**1,87 ± 0,50 ^{A}**
P1	2,20 ± 0,45 ^{aB}	2,80 ± 0,45 ^{bB}	1,80 ± 0,45 ^{aB}	**2,27 ± 0,50 ^{B}**
Mean	**1,80 ± 0,57^{a}**	**2,30 ± 0,71 ^{ab}**	**2,10 ± 0,42^{b}**	

Description; The distinction of lowercase superscripts alphabet within the same row appeared noteworthy contrasts (P<0.05), the contrast of uppercase superscripts alphabet within the same column appeared critical differences (P<0.05), P0; The control group was given only NaCl or without PRP, P1; The group of rats given 100 ul PRP injection.

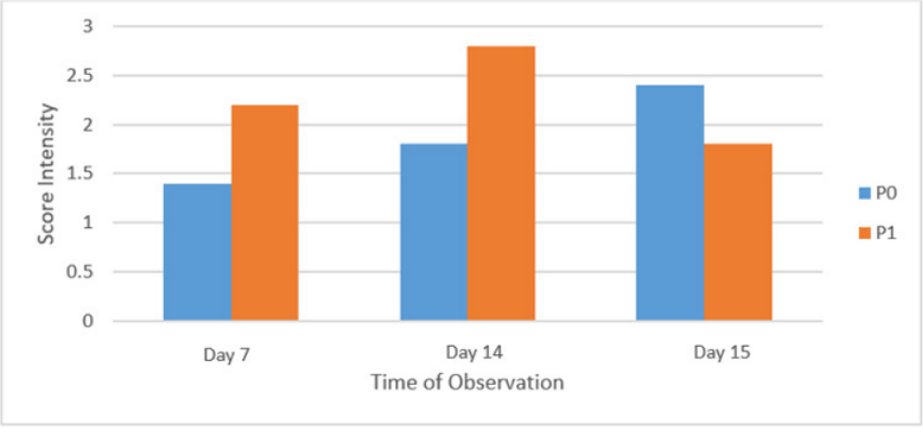

Figure 2. Histogram showed mean score of IS TGF- β1 expression in the Achilles tendon tissue of white rats (μm) in the P0 and P1 groups observed on the 7th, 14th and 21st days after tendon rupture.

DISCUSSIONS

The results of this study showed the intensity score (IS) distribution of TGF-β1 expression in the ruptured Achilles tendon tissue in the experimental group (P1) was faster than the control group (P0). The experimental group's intensity score showed the distribution of TGF - β1 expression increased on day 7th and peaked on day 14th, then decreased on day 21st. While in the control group (P0) increased time of observation started on day 7th, 14th and peaked on day 21st. The average intensity score showed the distribution of TGF- β1 expression in achilles tendon tissue of white rats with the experimental group on day 7th was higher (P<0.05) compared to the control group. The same result was seen on day 14th, the mean expression of TGF - β1 in the P1 group was higher (P<0.05) compared to the P0 group. However, on the 21st day of observation, the average expression of TGF- β1 in the experimental group was significantly lower (P<0.05) compared to the control group. These results confirmed that administration of 100 μl of autologous PRP to white rats with Achilles tendon rupture can increase TGF-β1 expression in ruptured tendon tissue at the beginning of the healing process. The results of this study were in line with studies conducted by Lyras *et al.* in rabbits with patellar tendon injuries given platelet-rich plasma showed a significant increased in the expression of TGF - β1 in the spatial and temporal tissues of the patellar tendon in the early stages of healing (1 to 4 weeks).[19]

Other results also shown a significant result (p<0.01) shown that TGF-β1 level in ELISA kit measurement was higher rather than MSTN in decorin 25ng/mL group, PrP 10% group, and also in PrP 10%+ decorin 25ng/mL group.[20]

The limitation of this study was that we cannot measure other parameters as a comparison to determine the effect of PrP. In addition, in this study the concentration of PRP administration did not vary so it was not possible to determine the effective dose of in vivo PRP administration to obtain the desired optimal effect.

CONCLUSION

Administration of Platelet Rich Plasma (PRP) can increase the intensity score of

TGF-β1 expression during the early phase healing process of Achilles tendon rupture in white rats (*Rattus norvegicus*).

DISCLOSURE

Funding
None.

Ethical Approval
The study was accepted from ethical approval in our institution.

Conflict of Interest
None.

Author Contributions
We declare that all of the authors contribute to article preparation.

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