Relationship of Cytokine Levels and Clinical Effect on Platelet-Rich Plasma-Treated Lateral Epicondylitis

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ABSTRACT: Lateral epicondylitis (LE) is difficult to manage and can result in significant patient morbidity. Currently, the clinical use of platelet-rich plasma (PRP) for painful tendons has received attention, but its efficacy remains controversial. This study aimed to investigate the clinical effects of PRP and its biological components. A total of 156 patients with LE were randomly divided into group 1, treated with a single injection of 2-ml autologous PRP, and group 2, treated with a control received only physical therapy without injection. Both groups used a tennis elbow strap and performed stretching and strengthening exercises during 24 weeks' follow-up. Pain and functional improvements were assessed using the visual analog scale (VAS), Modified Mayo Clinic Performance Index for the elbow, and magnetic resonance imaging (MRI). White blood cell count, platelet count, and levels of platelet-derived growth factor-AB (PDGF-AB), PDGF-BB, transforming growth factor-β (TGF-β), vascular endothelial growth factor, epithelial growth factor, and interleukin-1 β in PRP were measured and investigated for statistical correlation with the clinical score. At 24 weeks, all pain and functional variables, including VAS score, Mayo Clinic performance scores, and MRI grade, improved significantly in group 1 ($p < 0.05$). PDGF-AB, PDGF-BB, and TGF-β levels were more significantly increased in PRP than in whole blood. TGF-β level significantly correlated with Mayo Clinic performance score and MRI grade improvement. Thus, TGF-β level in PRP is considered to play a pivotal role in tendon healing. These results may contribute to identifying the best protocol for PRP application in tendinopathies. © 2017 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 36:913–920, 2018.

Keywords: platelet-rich plasma (PRP); lateral epicondylitis; sports medicine; growth factor; PRP

Lateral epicondylitis (LE; tennis elbow) is a common problem among patients whose activities require strong gripping or repetitive wrist movements. It is noteworthy in sports and occupational settings owing to repetitive trauma and overuse; besides, they are prevalent among individuals of all ages and part of the aging process.[1] The term *epicondylitis* suggests that this is an acute inflammatory condition; however, it is more aptly described as "tendinosis." The most commonly affected structure is the extensor carpe radialis brevis (ECRB), which attaches to the lateral epicondyle of the humerus. The chronic degenerative changes that occur in LE due to the repetitive strain injury implicated bear the hallmarks of tendinosis.

Numerous treatment modalities have been used for LE, of which the mainstay is nonoperative. Conservative treatment includes using non-steroidal anti-inflammatory drugs and corticosteroid injections, but their long-term effectiveness is controversial.[2] As a relatively novel autologous source, platelet-rich plasma (PRP) has been the focus in the field of orthopedic surgery. PRP, a so-called buffy coat product, is prepared from freshly collected autologous blood sample. It is a mixture of platelet- and leukocyte-rich plasma, activated with thrombin to produce a viscous gel cloth.[3] It contains a high concentration of platelets along with at least six abundant platelet growth factors within α-granules, each of which has a specific function during wound repair.[4,5] Many basic and clinical investigations concerning the therapeutic efficacy of PRP in sports-related injuries and disorders have been published.[6–12] In addition, athletes tend to choose treatments with little or no published peer-reviewed evidence of efficacy, mostly owing to the fact that it takes many years for new treatment modalities to be fully validated in large, prospective, randomized controlled trials.[13] Owing to these demands, PRP may be an attractive option for novel use by clinicians in sports medicine.

PRP is promoted as an ideal autologous biological blood-derived product that can be exogenously applied to various tissues, where it releases high concentrations of platelet-derived growth factors that enhance wound, the bone, and tendon healing. When platelets are activated, growth factors are released and initiate the natural healing response of the body. Platelets contain or activate many different cytokines such as platelet-derived growth factor AB (PDGF-AB), PDGF-BB, transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), interleukin 1 beta (IL-1b), and a growth factor for hepatocytes. These cytokines are involved in the healing of injured tendons[14–16] and regulate cellular processes such as chemotaxis, angiogenesis, mitogenesis, differentiation, and metabolism.[17] The rationale behind PRP therapy is that additional platelets will increase the amounts of multiple growth factors released to a localized injury site, which, in turn, will augment the healing process in injured tissues. In a previous study, after 1 year of elbow tendinosis treatment with PRP or with corticosteroids,

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73% of the patients in the PRP-treated group had improved scores in the visual analog scale (VAS) for pain and disabilities of the arm, shoulder, and hand when compared with 50% of the patients in the corticosteroid group.[18] These studies suggest that in addition to its stimulatory effects on the repair of injured tissues, PRP can reduce tendon inflammation, which is associated with pain. Nevertheless, this study and most of the other clinical studies lack quantification of the associated clinical effects and actual components of PRP after injection.

Considering the increasing attention given to PRP treatment for LE, the clinical effect of the cytokine composition of PRP must be investigated to encourage and assist physicians to use it safely and efficiently. This could also lead to robust clinical research for defining the scope of use of PRP and its anticipated effects.

MATERIALS AND METHODS

Patient Enrollment

In this single-center, prospective, randomized controlled trial, the enrolled patients with LE were either transferred by other physicians or included those who saw the information on the bulletin board at Chosun University Hospital. These patients were screened for eligibility based on the following diagnostic criteria: (i) aggravation of the lateral elbow pain during wrist extension and relief at rest; (ii) tenderness of the lateral epicondyle; and (iii) positive result in Cozen's test. However, the inclusion criteria were as follows: (i) unilateral elbow pain for >3 months; (ii) LE diagnosed on magnetic resonance imaging (MRI); (iii) no improvement in the condition despite receiving treatment in the previous 3 months. We excluded patients who had central or peripheral nervous system diseases, hematological diseases (e.g., platelet dysfunction syndrome, or platelet count <100,000/μl), radial nerve entrapment, inflammatory diseases, gout, tumor, or radiocapitellar osteoarthritis; underwent operation for lateral epicondylalgia; or were pregnant. After screening, the patients were randomly assigned computer-generated numbers and then divided into a PRP-treated group and a manipulation group as controls. Patient information was encoded by another researcher who did not participate in data analysis. Informed consent was obtained from all the participants orally and in writing. This study was performed in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki. All the patients signed a written informed consent explaining the potential benefit of PRP, surgical procedure, and follow-up. The protocol of this trial is approved by the Chosun University Hospital, GwangJu, South Korea (institutional review board No. 2014-08-005).

Pain Intensity

Pain severity was evaluated by using the VAS (0, most satisfactory to 100, poor) before injection, and reevaluation was performed at 3 and 6 months after the injection. The validity and reliability of self-rating scales such as the VAS has been described previously.[19,20] $-\Delta$VAS was represented by the calculated $-(\mathrm{VAS_{final}} - \mathrm{VAS_{first}})$ in accordance with the VAS score at the first and final visit of the patient.

Functional Outcome Measures

The Modified Mayo Clinic Performance Index for the elbow was used as a valid and reliable parameter to evaluate the functional improvement after therapy.[21] The Mayo Clinic Performance Index for the elbow has four parameters, namely pain, motion, stability, and daily function. The maximum index score is 100, and the minimum index score is 0. The results are interpreted as excellent (≥90), good (75–89), fair (60–74), and poor (<59). The pain parameters in this questionnaire carry the highest points, which is 45 of 100. The modified Mayo questionnaire was specific to changes in elbow function. The questions were found to be reliable, reproducible, and sensitive to change in elbow function. Its construct validity is good for patient-rated variables and excellent for physician-rated variables. A minimal clinically important difference of 15 was reported for patients with rheumatoid arthritis after arthroplasty or synovectomy. The Mayo questionnaire was filled out via interviews with the patients before and after therapy. ΔMAYO was calculated as $\mathrm{MAYO_{final}} - \mathrm{MAYO_{first}}$ by measuring MAYO score at the first and final visits of the patient.

PRP Preparation

Peripheral venous blood sample is collected into three 9-ml tubes containing 3.8% (wt/vol) sodium citrate. WBC and platelet counts were counted at Chosun University Hospital. Anticoagulated blood sample was centrifuged at 1,200 rpm for 6 min, and PRP was collected using by commercial kit (HUONS, Sungnam, Korea), taking care to avoid contamination with the buffy coat containing the leukocytes. Plasma sample was kept at room temperature until intervention; the delay between blood extraction and plasma administration should not be >4 h. Just preceding PRP administration, 10% calcium chloride was added up to a final concentration of 22.6 mM (50 μl/ml of PRP), and a 5-ml Luer Lok syringe was filled with the activated PRP.

Procedures

Interventions were performed by two radiologists with extensive clinical experience in musculoskeletal intervention procedures. An exploratory echography was performed to identify clefts of hypoechogenicity and/or changes in vascularity, and baseline sonographic characteristics were recorded.

Needle Tenotomy with PRP

Ultrasonography-guided percutaneous needle tenotomy with PRP was performed only once at the beginning of the study. Blood samples were drawn from all the patients' unaffected arm, and PRP was prepared as described earlier. By using a single skin portal, a local anesthetic (2 ml of 1% lidocaine HCl 10 mg/ml) was injected in the subcutaneous tissue of the lateral elbow by using a 20-G needle. Once the needle was in place, the 5-ml Luer Lok syringe loaded with the treatment was attached. And, we injected local anesthetics only on the subcutaneous tissues and changed the syringe and injected PRP onto the tendon.

Growth Factor Quantification Assays

The PRP samples (100 μl) were prepared immediately after centrifuge at 4°C for analysis of growth factor content. Commercially available enzyme-linked immunosorbent assay kits (Quantikine ELISA Kit, R&D Diagnostics, Wiesbaden, Germany) were used in accordance with the manufacturer's

instructions to quantify the concentrations of PDGF-AB, PDGF-BB, TGF-β, VEGF, EGF, and IL-1b. All growth factor measurements were performed in duplicates, and no unexpected scattering of data (<10%) was observed. Absorbance at 450 nm was read by using a microtiter plate reader, and the concentration of the growth factors were calculated by comparing with the standard curve, respectively.

Outcome Assessment

Pain relief was the primary outcome of the trial, while improvement in functional impairment and grip strength were regarded as the secondary outcomes. For pain assessment, we used the VAS scores (0, most satisfactory to 100, poor) for recording changes each time before the treatment procedure in three states, namely rest, daily activity, and work situations, from the beginning up to 8 weeks of the study.

Follow-Up MRI

MRI was performed at the first visit of the patient and at 6 months' follow-up by using a 1.5-Tesla MR system (Avanto, Siemens Medical Solutions, Erlangen, Germany). MRI examination was performed in the supine position of the elbow and palms and elbow extension. The signal intensity within the common extensor was evaluated on T2-weighted images. For MRI assessment, we described a new grade classification of the severity of common extensor tendinopathy that was a modification of the system devised by Walz et al.[22] In the grading system, complete, homogenous, low signal intensity in the common extensor tendon was grade 0; focal increased signal intensity (signal change of less than one-half of the tendon width), grade 1; moderate focal increased signal intensity (signal change of more than one-half of the tendon width), grade 2; and generalized increased signal intensity, grade 3; the histologic findings well correlated with the MR imaging features of tendon degeneration and the degree of tendon tear.[23] All the magnetic resonance images were interpreted by a musculoskeletal radiologist who was blinded to all clinical information and the severity of the disease. $-\Delta$MRI was calculated as $\mathrm{MRI_{final}} - \mathrm{MRI_{first}}$ by measuring the MRI grade at the first and final visits of the patient.

Statistical Analysis

All the quantitative measurements were described by using summary statistics (n, mean, standard deviation, Pearson Correlation coefficient, and p value). WBC count, PLT count, respective growth factor level, $-\Delta$VAS, and ΔMAYO were compared by using the sign test for nonparametric, paired data. The Pearson correlation coefficient was used to demonstrate the relationship between the scores and the PRP growth factor levels. In the biological examination, statistical analysis was performed by using the Student t test or analyzed by using one-way analysis of variance. The groups were compared by using the Tukey multiple comparison test, and p values of <0.05 were considered significant.

RESULTS

Clinical Assessment of the PRP-Treated Group

In the present study, the patients who had a history of lateral epicondyle pain over the previous 3 months and were not treated with other injections were enrolled. Of the 156 subjects who met the inclusion criteria, 36 were excluded and 15 lost to follow-up from the study (Fig. 1). The characteristics of the remaining 105 subjects are presented in Table 1. No significant differences were found between the groups in terms of age, sex, hand dominance, intervention on the dominant extremity, preoperative VAS score, Mayo elbow score, MRI grade, and follow-up duration ($p > 0.05$). No complications were noted in either group at the treatment period. In particular, no infections, neurovascular complications, or worsening of the patients' lateral elbow pain was observed.

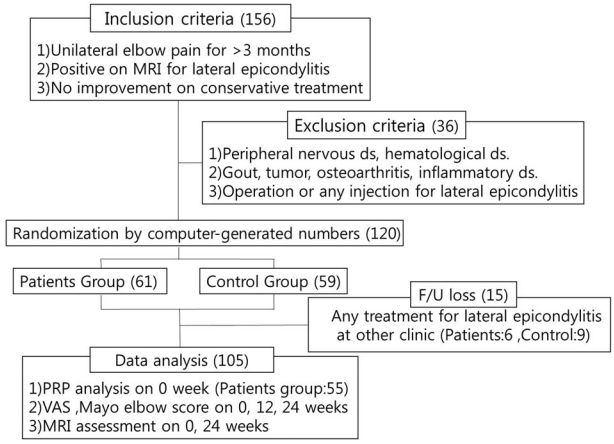

Figure 1. A flow diagram for patient enrollment or exclusion in the study.

To investigate the effect of PRP treatment on the clinical aspect of LE, the differences in $-\Delta$VAS, ΔMAYO, and $-\Delta\mathrm{M_{Grade}}$ were calculated after PRP treatment and compared with those of the control. Four weeks after the procedures, the PRP group reported an improved score of 40.6 in their $-\Delta$VAS compared with the 29.2 in the control group (Fig. 2a). Moreover, ΔMAYO had improved to 16.23 in the PRP group compared to 8.42 in the control (Fig. 2b). Finally, $-\Delta\mathrm{M_{Grade}}$ showed a 1.11 grade improvement in the PRP group compared to 0.37 in the control (Fig. 2c).

Relationship Among WBC Count, Platelet Count, Cytokine Levels, and Clinical Scores

To investigate the biological elements that led to the clinical improvement of LE, WBC count, platelet count, and general cytokines, which are known to play a critical role in LE improvement, such as PDGF-AB, PDGF-BB, TGF-β, VEGF, EGF, and IL-1β, were measured in whole blood and PRP samples for treatments. In the PRP preparation for injection, WBC count increased to 41.9×10^9/l from 6.2×10^9/l, and platelet count increased to 18.9×10^7/l from 2.75×10^7/l (Fig. 3a). Among the cytokines, PDGF-AB, PDGF-BB, and TGF-β levels significantly increased in PRP. In particular, the TGF-β level increased from 3.92 to 112 ng/ml (Fig. 3b). To identify the component in PRP that had the greatest influence on LE improvement when PRP treatment was administered, the Pearson correlation coefficient was used to analyze the relationship between $-\Delta$VAS and

Table 1. Comparative Demographics of PRP Versus Control Group

	No. or Average (Range)	
No. of Patients	PRP	Control
Sex		
M	29	26
F	32	33
Age	50.12941	54.49153
Dominance		
Right hand	43	40
Left hand	42	19
Ambidextrous		
Initial Visual Analog Pain Score	64.27059	44.88136
Initial Mayo Elbow Score	66.76471	75.64407
Initial MRI grade	1.811765	1.474576
Follow-up duration, weeks	3 month	3 month
Follow-up loss	6	9
Enrolled patients	55	50

ΔMAYO, and the PRP components, respectively (Table 2, Fig. 4ab). The Pearson correlation coefficient between −ΔVAS and WBC count was 0.318 ($p = 0.018$), and that between ΔMAYO and TGF-β was 0.275 ($p = 0.042$).

Relationship Between MRI Assessment Result and Growth Factors in PRP

To investigate the effects of the respective blood components, including cytokines, on the changes in MRI grade, WBC count, platelet count, and levels of PDGF-AB, PDGF-BB, TGF-β, VEGF, EGF, and IL-1β were presented on $-\Delta M_{G0}$, $-\Delta M_{G1}$, $-\Delta M_{G2}$, and $-\Delta M_{G3}$, respectively (Fig. 5). No change in MRI grade denotes $-\Delta M_{G0}$ and one, two, three for $-\Delta M_{G1}$, $-\Delta M_{G2}$, $-\Delta M_{G3}$, respectively. Among the growth factors, TGF-β and VEGF levels sequentially increased significantly according to the increase in −ΔM.

MRI Assessment

PRP was injected to a 45-year-old female patient. Before the treatment, coronal MRI showed LE of grade II (Fig. 6). At 6 months' follow-up after the PRP injection, MRI revealed a decrease in signal intensity at the origin of the ECRB. In addition, the patient's Mayo performance (32–65) and VAS clinical scores (90–20) improved.

DISCUSSION

LE, also known as tennis elbow, remains one of the most perplexing disorders of the musculoskeletal system. It is thought to result from overuse or repetitive microtrauma resulting in a primary tendonosis of the ECRB muscle with or without involvement of the extensor digitorum communis (EDC) and extensor carpi radialis longus (ECRL).[24] During the last decade, various conservative and noninvasive treatments have been used without consistent and satisfactory results, and a transition to the use of PRP injection for treatment of LE was observed. PRP injection has advantages, including being a bioactive component of whole blood, which is composed of many growth factors and cytokines. Especially, a recent systematic review suggests that PRP with leukocyte-rich formulations were better for tendonopathy.[25] Increasing the local regenerative stimulus for tendon healing is thought to be the main advantage of PRP application, but this has not been substantiated.[26] Owing to the controversy regarding release and debridement with pain relief, we wished to compare PRP injection and physical therapy as a control treatment in patients with LE in terms of (i) residual pain at the end of follow-up; (ii) functional improvement; and (iii) MRI grade. In the present study, −ΔVAS (pain score), ΔMAYO (function score), and $-\Delta M_{Grade}$ (MRI grade) significantly improved in the PRP group. Mishra and Pavelko compared the effectiveness of leukocyte-enriched PRP to standard corticosteroid treatment for LE and found that at short-term follow-up both groups showed significant improvement in pain and function, but over long-term follow-up, pain and function scores returned to baseline for corticosteroid group and remained high for the PRP group.[27,28] Also, several other large studies that used single injection of inactivated PRP on chronic lateral epicondylitis could reduce pain and increase function significantly, exceeding the effect of corticosteroid injection even after a follow-up of 2 years.[29] Therefore, local injection of PRP possibly offers significant symptomatic relief and PRP may be regarded as a better treatment option.

PRP is known to be an ideal autologous biological blood-derived product that releases high concentrations of platelet-derived growth factors on injection,

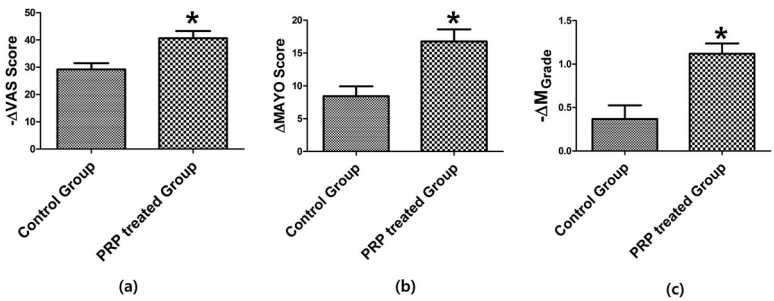

Figure 2. Clinical aspect between the control and PRP-treated groups. (a) −ΔVAS is calculated as $-(VAS_{final} - VAS_{first})$ by using the VAS scores at the first and final visits of the patient. (b) ΔMAYO is calculated as $(MAYO_{final} - MAYO_{first})$ by using the Mayo performance scores at the first and final visits of the patient. (c) −ΔMRI is calculated as $-(MRI_{final\ grade} - MRI_{firs\ grade})$ by using the MRI grades at the first and final visits of the patient.

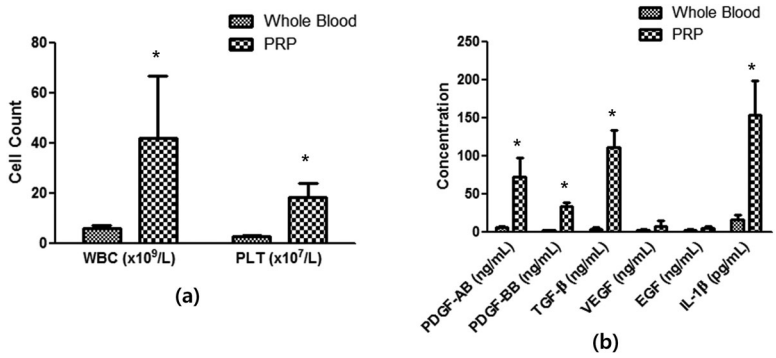

Figure 3. Biological components in whole blood and PRP samples. (a) WBC and PLT counts. (b) Growth factor concentration. Data in the bar graphs are expressed as mean ± standard deviation. $^{*}p < 0.05$.

Table 2. Corelation Coefficiency of WBC, PLT, and Growth Factors on −ΔVAS or ΔMAYO in PRP-Treated Group

		WBC	PLT	PDGF-AB	PDGF-BB	TGF-β	VEGF	EGF	IL1b
−ΔVAS	Pearson correlation coefficiency	0.318*	0.165	−0.011	−0.008	0.176	−0.036	−0.148	0.014
	p value	0.018	0.229	0.936	0.954	0.199	0.795	0.284	0.921
	N	55	55	55	55	55	55	55	55
ΔMAYO	Pearson correlation coefficiency	0.168	0.152	−0.128	0.055	0.275*	−0.091	−0.115	−0.007
	p value	0.221	0.269	0.352	0.699	0.042	0.510	0.407	0.959
	N	55	55	55	55	55	55	55	55

$^{*}p < 0.05$.

which enhance tendon healing due to its effects on angiogenesis and collagen synthesis.[30] Various growth factors and cytokines in PRP include platelet-derived growth factors (PDGF-aa, PDGF-bb, PDGF-ab), TGF-β (TGF-b1, TGF-b2), fibroblast growth factor (FGF), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2, respectively), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), interleukin 8 (IL-8), keratinocyte growth factor, and connective tissue growth factor. In the present study, WBC and platelet counts significantly increased by 6.76- and 6.87-fold in PRP, respectively. In addition, PDGF-AB, PDGF-BB, and TGF-β levels significantly increased 13-, 22-, and 28.6-fold in whole blood. Platelets release >95% of presynthesized growth factors within 1 h of activation.[31] This initial burst is followed by steady synthesis and secretion of growth factors for their remaining life span, and PDGF-AB, PDGF-BB, and TGF-β in injected PRP could be considered to be activated immediately.

The main finding of this study is the correlation of clinical assessment to the follow-up and biological components of PRP. PRP injection resulted in better pain control, and the functional improvement was stable and maintained up to follow-up. Therefore, the cytokines whose levels were increased in correlation with −ΔVAS (pain relief to follow-up), ΔMAYO (functional improvement), and −ΔMRI grade were investigated. Among them, TGF-β was highly correlated with ΔMAYO and −ΔMRI grade. Several studies have addressed the relationship between neuropeptides and cytokines because neuropeptides are probably potent stimulators of proinflammatory cytokine generation. However, no research has been conducted about the relationship between cytokines and clinical relevance. Our study demonstrates a

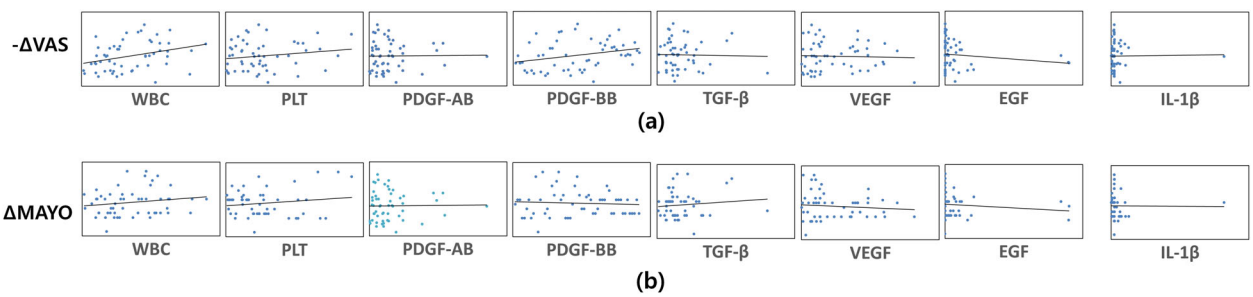

Figure 4. Pearson correlation analysis between the clinical score and the biological components. (a) Correlation between −ΔVAS and the biological components. (b) Correlation between ΔMAYO and the biological components.

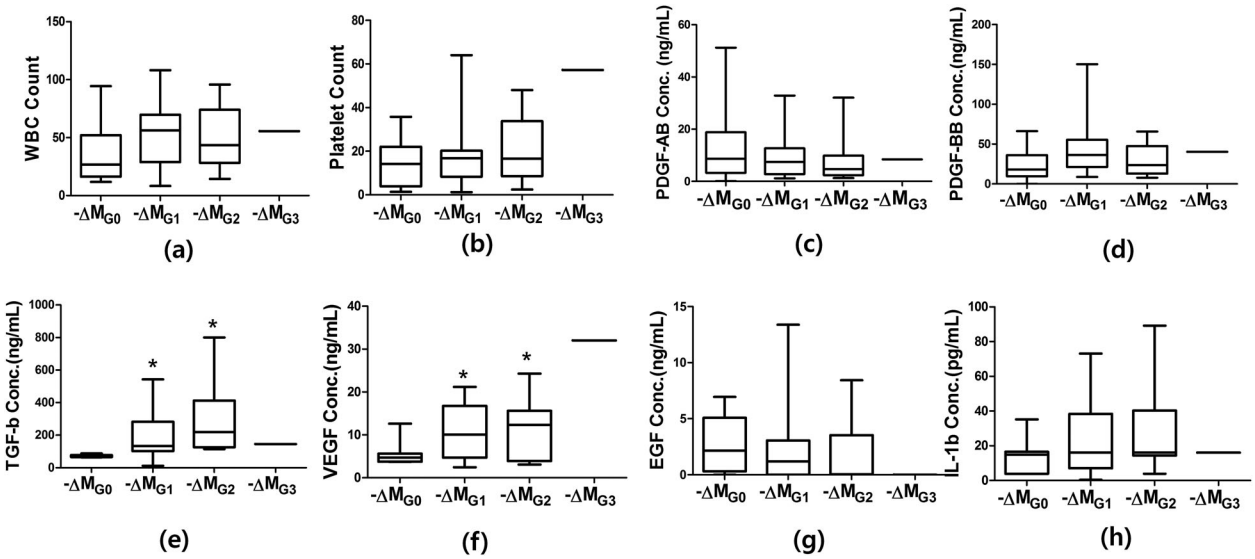

Figure 5. WBC count, PLT count, and growth factor concentration according to −ΔMRI grade. (a) WBC count at the respective changes in MRI grade; (b) platelet count; (c) PDGF-AB concentration; (d) PDGF-BB, (e) TGF-b, (f) VEGF, (g) EGF, and (h) IL-1b. Data in the bar graphs are expressed as mean ± standard deviation. $^{*}p < 0.05$.

significant positive correlation between TGF-β and ΔMAYO ($p = 0.042$ and $r = 0.275$) after PRP treatment with Pearson's correlation analysis. In addition, a positive correlation was found between TGF-β, VEGF, and −ΔMRI grade, and the patient who received a PRP injection that included a high concentration of TGF-β showed improved MRI finding. TGF-β is a pleiotropic cytokine with potent regulatory and inflammatory activities.[32] The multi-faceted effects of TGF-β on numerous immune functions are cellular- and environmental-context dependent.[33] Especially, it was reported that PRP including rich TGF-β stimulated cell proliferation and total collagen production in the human tenocyte cultures.[34] TGF-β binds to TGF-β receptor II (TGF-βRII), triggering the kinase activity of the cytoplasmic domain that in turn activates TGF-βRI. The activated receptor complex leads to nuclear translocation of Smad molecules, and transcription of target genes. In the inflammatory condition, TGF-β in the presence of IL-6 drives the differentiation of T helper 17 (Th17) cells, which can promote further inflammation and augment autoimmune conditions. TGF-β orchestrates the differentiation of both Treg and Th17 cells in a concentration-dependent manner. In addition, some reports said that VEGF release was highly increased by TGF-β-induced vascular smooth muscle cells.[35] TGF-β in combination with IL-4, promotes the differentiation of IL-9- and IL-10-producing T cells, which lack suppressive function and promote tissue inflammation. Based on our data on growth factor levels and these findings, we suggest that PRP improves LE by TGF-β-mediated mechanisms.

In this study, we performed only manipulation without needle tenotomy and injection of something (whole blood or saline) in the control group. Most patients in the clinic receive injections or only exercise and this would be limitation of present study. For precise study, it would be nice to have needle tenotomy and injection of something (whole blood or saline) in

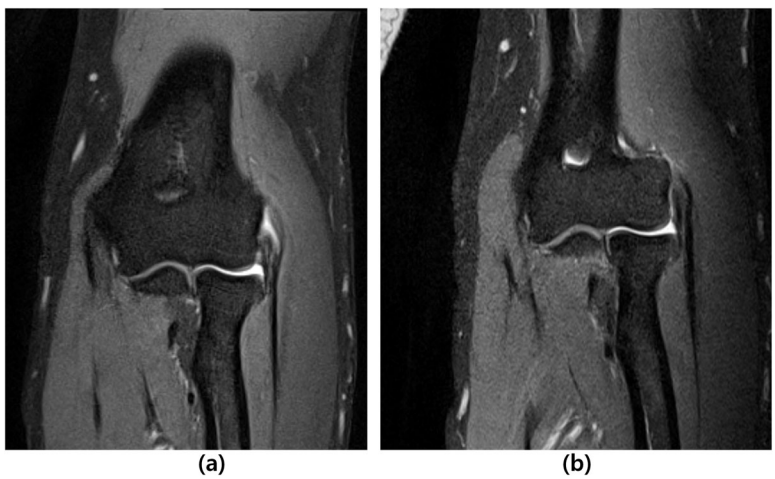

Figure 6. PRP injection in a 45-year-old female patient. (a) Coronal MRI scan before treatment, showing lateral epicondylitis grade 2. (b) MRI scan at 6 months' follow-up after PRP injection, showing decreased signal intensity at the origin of ECRB and improvement in the patient's Mayo performance (from 32–65) and VAS clinical scores (from 90–20).

the control group. In addition, other unanalyzed cytokines and inactivated PRP may contribute to the improvement of symptoms of lateral epicondylitis, so additional studies are needed to investigation of other cytokines and inactivated PRP.

Taken together, this report demonstrates that a single injection of concentrated autologous platelets improves pain and function in chronic LE. These improvements sustained for >6 months of follow-up, with no reported complications. Furthermore, our results revealed that TGF-β level, Mayo performance score, improvement of MRI grade had significant positive correlations, and this supports that TGF-β in PRP might play a role in the treatment of LE.

AUTHORS' CONTRIBUTIONS

Conception and design by Wonbong Lim and Young Lae Moon. Development of methodology by Wonbong Lim. Acquisition of data by Bora Kim. Analysis and interpretation of data (e.g., statistical analysis, biostatistics, and computational analysis by Wonbong Lim and Sangha Park. Writing and review of the manuscript by Wonbong Lim and Young Lae Moon. Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases) by Bora Kim, Sin Wook Kang, Jung Woo Lee, and Sangha Park. Study supervision by Young Lae Moon. All authors have read and approved the final submitted manuscript.

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