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ORIGINAL ARTICLE

Comparison of Effects of Leukocyte-Rich and Leukocyte-Poor Platelet-Rich Plasma on Pain and Functionality in Patients With Lateral Epicondylitis

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ABSTRACT

Objectives: This study aims to compare the effect of leukocyte concentration in platelet-rich plasma (PRP) on pain, functionality and post-injection local inflammatory reactions in patients with lateral epicondylitis.

Patients and methods: The study included 90 patients (26 males, 64 females; mean age 38.6 years; range 18 to 75 years) with lateral epicondylitis-related pain visual analog scale (VAS) score of ≥5 for more than three months. Patients were randomly assigned into three groups. Normal saline (1.5 mL) was injected in group 1 (control group) while a single dose of leukocyte-poor-PRP (1.5 mL) and leukocyte-rich-PRP (1.5 mL) were injected in groups 2 and 3, respectively. An exercise program was recommended to patients in all three groups. Patients were assessed according to VAS, Patient-Rated Tennis Elbow Evaluation, grip dynamometer and pinchmeter, extensor tendon thickness and cortical derangement at baseline and at fourth and eighth weeks after therapy. All patients were questioned regarding paracetamol use and adverse effects after therapy.

Results: No significant differences were detected between groups regarding VAS, Patient-Rated Tennis Elbow Evaluation, grip and pinch measurements, extensor tendon thickness and cortical derangement ($p>0.05$). In intra-group comparisons, VAS and Patient-Rated Tennis Elbow Evaluation scores obtained at fourth and eighth weeks were significantly decreased in all groups when compared to baseline values ($p>0.05$). Again, there was no significant difference in the control visit at eighth week when compared to baseline. Assessment of grip and pinch measurements revealed that values obtained at fourth and eighth weeks were significantly increased compared to baseline in all three groups ($p<0.05$). In leukocyte-rich-PRP group, a significant increase was detected in the values obtained at eighth week compared to those obtained at fourth week, but no significant change was detected in other groups. No significant difference was detected in extensor tendon thickness in any group. No significant difference was detected between groups in terms of paracetamol use and post-injection reactions.

Conclusion: According to our study findings, lateral epicondylitis does not seem to affected either leukocyte-rich-PRP or leukocyte-poor-PRP on pain and function in the short term. Leukocyte concentration had no association with post-injection local inflammatory reactions.

Keywords: Lateral epicondylitis; platelet-rich plasma; tennis elbow.

Lateral epicondylitis, also known as tennis elbow, is the most commonly diagnosed cause of pain at lateral aspect of elbow.[1] Tennis elbow is considered as an overuse injury of extensor tendons with repetitive micro-trauma.[2]

Various treatment modalities have been suggested for lateral epicondylitis including resting, non-steroidal antiinflammatory drugs, physical therapy, extracorporeal shock wave therapy, ultrasound therapy, botulinum injection, and corticosteroid injection. Surgical release may be needed in recalcitrant cases. Injection of biological agents such as platelet-rich plasma (PRP) achieves a favorable long-term clinical outcome.[3]

Platelet-rich plasma is an autologous blood product which contains concentrated platelets. Experimentally, PRP promotes tendinous healing through release of different growth factors. This

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treatment can optimize healing in pathological human tendons.[4]

There is no standard technique for the preparation of PRP. It can be prepared manually and there are also various commercial PRP preparation kits approved by United States Food and Drug Administration.[5,6]

In average, PRP has three- to five-fold higher platelet count compared to whole blood. There are no widely accepted and quantified values of platelet concentrations in PRP.[5,6] There are four types of PRP; each of them has two subtypes based on the platelet and leukocyte counts as well as activation of PRP (Table 1). The use of leukocyte-rich (type 1) PRP (LR-PRP) may produce a more intense local inflammatory response and be associated with an increased post-injection pain response.[7-10] In this study, we aimed to compare the effect of leukocyte concentration in PRP on pain, functionality and post-injection local inflammatory reactions in patients with lateral epicondylitis.

PATIENTS AND METHODS

This double-blinded, randomized, controlled study comparing the effects of LR (Table 1: type 1A) and leukocyte-poor PRP (LP-PRP) (Table 1: type 3A) on function and pain in patients with lateral epicondylitis was conducted at Erciyes University between 15 February 2015 and 15 June 2015.

The study was conducted on 90 patients (26 males, 64 females; mean age 38.6 years; range 18 to 75 years) who were suffering from pain associated with lateral epicondylitis for more than three months (visual analog scale [VAS] ≥5). Pregnant females, patients with cervical radiculopathy or carpal tunnel syndrome, peripheral nerve injury, thrombocytopenia or other coagulation disorder, those receiving anticoagulant therapy, those with systemic diseases such as diabetes, rheumatoid arthritis or other inflammatory arthritis (such as psoriatic arthritis, ankylosing spondylitis), hepatitis, those who underwent steroid injections within three months prior to the study and those who received physical therapy and rehabilitation program for lateral epicondylitis within six months prior to the study were excluded.

The study protocol was approved by the Erciyes University Ethics Committee. A written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The diagnosis of lateral epicondylitis was primarily made based on patients' history and clinical examinations. Patients with at least one positive test (Mill's test, Cozen's test, Maudsley test) were included. Erythrocyte sedimentation rate, C-reactive protein, complete blood count and biochemistry tests were ordered from the patients. Plain radiographs of lateral epicondylitis were usually normal. Pre-arch and lateral elbow radiographs were performed to demonstrate soft tissue calcifications and establish a differential diagnosis.

Patients were randomly assigned into three groups (n=30 in each) by using the block randomization method. The treatment was performed by another investigator who had four years of experience. Both the patient and investigator were blinded to the treatment given. Normal saline (1.5 mL) was injected to the patients in group 1 (control group) while LR-PRP (1.5 mL) and LP-PRP (1.5 mL) were injected to the patients in groups 2 and 3 via peppering technique at the most sensitive point, respectively. Peppering technique involves insertion of a needle into the tendon to inject some of the blood; followed by retraction of the needle to subcutaneous tissue and redirection and reinsertion of the needle. In this study, the injections were performed without ultrasound guidance.

Manual PRP preparation techniques were used in this study. According to the system instructions, blood (16 mL) was drawn from cubital vein; then, citrate (4 mL) was added to prevent premature clotting. Following placement of blood samples in 10 mL tubes, the LP-PRP was centrifuged at 1075 rpm for 15 minutes in the first step. Then, it was re-centrifuged at 1495 rpm for 15 minutes under layer 1 and layer 2 of laminar flow. Following centrifugations, the platelets were initially collected by a poor plasma syringe and these platelets were not used for injection. Thereafter, the remaining platelets were collected from the tube and two aliquots containing approximately 1.5 mL of PRP were obtained. Of these, one was injected to the patient while the other was used to determine

cell count. After receiving the same amount of blood for LR-PRP, the blood was centrifuged at 1190 rpm for 20 minutes in the first step. Then, it was re-centrifuged for 15 minutes at 1890 rpm in the second step. The platelet concentration was intended to be two- or eight-fold higher than the basal value while the leukocyte concentration was intended to be three-fold lower and three-fold higher than the basal value in the LP-PRP and LR-PRP groups, respectively.

In all groups, an exercise program was prescribed including three sets per day consisting of 10 repetitive range of motion, stretching, strengthening, and gripping exercises for over four weeks. The exercise program was initiated on the second day after injection. The patients were given brochures containing a written statement. Patient compliance of the exercise program was recorded.

Non-steroidal antiinflammatory drug use was prohibited for one week before and after PRP application. The use of paracetamol up to 4 g/day for pain before and after injection was allowed.

At baseline and on the fourth and eighth weeks after treatment, the patients were evaluated by using VAS, patient-based forearm questionnaire, flu dynamometer and pinch measurements, extensor tendon thickness and cortical irregularity. Jamar type Saehan brand (Saehan Corp. Masan, Korea) hydraulic hand dynamometer was used to assess the maximum gripping force, which was recorded as libra unit. A hydraulic pinchmeter same brand as hand dynamometer was used to evaluate finger grip strength. The extensor tendon thickness was calculated by measuring the maximum thickness in the sagittal plane using a superficial linear probe with a Toshiba Aplio 500 (Toshiba Co. Ltd. Tokyo, Japan) sonography device by the same physician blinded to the treatment. The patients were questioned for paracetamol use and side effects (swelling, redness, and/or soreness) after treatment.

Statistical analysis

Data were analyzed using the IBM SPSS Statistics software version 22.0 (IBM Corp., Armonk, NY, USA). The normality of the data was examined by Shapiro-Wilk normality test and Q-Q graphs. The normality of all numerical variables was re-examined using the D'Agostino-Pearson test. As a result, it was verified that the variables are not normally distributed. Numerical variables were compared by using Kruskal-Wallis test. On the other hand, intra-group comparisons were performed by using the Friedman test. Categorical variables were compared by using Fisher's exact test. A p value <0.05 was considered statistically significant.

RESULTS

In the control group, the dominant arm was affected in 16 of 27 patients with right arm dominance and 21 of 30 patients with right arm dominance in LP-PRP and LR-PRP groups, respectively. Table 2 presents data distribution of demographic characteristics.

In this study, no significant difference was found between the groups regarding visual analog scale, patient-based forearm assessment questionnaire, daily life questionnaire, flu and pinch measurements, extensor tendon thickness and cortical irregularity ($p>0.05$). In intragroup comparisons, visual analog scale and patient-based

Table 1. Types of platelet-rich plasma

	White blood cells	Activation of PRP	Concentration of platelets
Type			
1A	Increased	No	≥5×
1B	Increased	No	<5×
2A	Increased	Yes	≥5×
2B	Increased	Yes	<5×
3A	Minimal or absent	No	≥5×
3B	Minimal or absent	No	<5×
4A	Minimal or absent	Yes	≥5×
4B	Minimal or absent	Yes	<5×

PRP: Platelet-rich plasma.

Table 2. Distribution by demographic characteristics

	Control group					LP-PRP group					LR-PRP group					p
	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max	
Age (year)			47.6±9.1	46.50	30-67			45.0±8.6	46.00	28-60			46.5±8.7	45	21-67	0.505
Sex																0.089
Woman	19	63.3				26	86.7				19	63.3				
Male	11	36.7				4	13.3				11	36.7				
Affected arm																0.343
Right	16	53.3				21	70.0				21	70.0				
Left	14	46.7				9	30.0				9	30.0				
Dominant arm																0.104
Right	27	90.0				30	100.0				30	100.0				
Left	3	10.0				0	0.0				0	100.0				

LP-PRP: Leukocyte-poor platelet-rich plasma; LR-PRP: Leukocyte-rich platelet-rich plasma; SD: Standard deviation; Min: Minimum; Max: Maximum.

forearm evaluation questionnaire scores were significantly lower on the fourth and eighth weeks after injection when compared to baseline in all groups ($p<0.05$). No significant decrease was observed on the eighth week when compared to the results obtained on the fourth week after injection (Table 3).

When the grip and pinch measurements were evaluated, significant improvement was detected on the fourth and eighth weeks when compared to baseline in the PRP and control groups ($p<0.05$). In addition, a significant improvement was observed on the eighth week when compared to the fourth week after injection in LR-PRP group. There was no significant increase in other groups. When the extensor tendon thickness was assessed, no significant reduction was observed in any group. There was no difference in paracetamol use and post-injection reactions (swelling, redness, and/or soreness) among groups.

DISCUSSION

Our study indicated that single-dose LP-PRP or LR-PRP is not more effective than normal saline on pain and function in patients with lateral epicondylitis. There are conflicting results regarding the effectiveness of PRP in the literature. In the study conducted by Montalvan et al.,[11] 25 patients were assigned into two groups in which PRP were injected twice over a period of four weeks in the study group and normal saline was injected in the control group. Patients were evaluated for VAS on first, third, sixth, and 12th months. In that study, the pain scores improved significantly at the sixth and 12th months in both groups but no significant difference was detected among groups. However, in another study conducted by Mishra et al,[7] 116 of 230 patients were assigned into PRP group. The remaining patients were assigned into the control group. Both study and control groups were evaluated on the 12th and 24th weeks. Authors found that there was no significant difference in VAS scores on the 12th week between groups due to resistant wrist extension, but significant improvement was observed in LR-PRP group compared to control group on the 24th week. These results brought the question: "Can PRP be more effective in the long term?" This may explain the contradictory results

Table 3. Comparison of visual analog scale scores across groups

	Control group			LP-PRP group			LR-PRP group			
	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	*p*
VAS (nocturnal) before therapy	6.8±1.8	7	3-10*	7.4±2.1	8	3-10*	6.5±2.4	7	0-10*	0.269
VAS (night) 4th week	4.2±2.5	4.5	0-8†	3.7±3.0	4	0-9†	4.5±3.0	5	0-10†	0.603
VAS (nocturnal) 8th week	3.47±3.00	4.5	0-10†	3.4±3.0	3	0-9†	3.2±3.4	2	0-10†	0.903
*p**		<0.001			<0.001			<0.001		
VAS (motion) before therapy	8.2±1.7	9	5-10*	8.7±1.7	10	4-10*	7.9±1.8	8	3-10*	0.100
VAS (motion) 4th week	5.9±3.2	6	0-10†	5.6±2.6	5	2-10†	5.8±2.7	5	0-10†	0.857
VAS (motion) 8th week	5.3±3.1	5	0-10†	5.2±2.9	5	0-10†	4.3±3.2	3.5	0-10‡	0.307
*p**		<0.001			<0.001			<0.001		

LP-PRP: Leukocyte-poor platelet-rich plasma; LR-PRP: Leukocyte-rich platelet-rich plasma; VAS: Visual analog scale; SD: Standard deviation; Min: Minimum; Max: Maximum; * before therapy min-max values; † 4th week min-max values; ‡ 8th week min-max values.

due to the short duration of our work. It should also be kept in mind that the lack of standard PRP preparation technique and the absence of platelet and leukocyte concentration may still affect the results.

In our study, there were no significant differences between the groups despite significant improvements in functional measures. Sixty patients were included to a double-blind random-controlled study by Krogh et als.[12] These patients were divided into three groups to receive PRP, saline or steroid injection. The patient-based forearm questionnaire scores of the patients were evaluated on the first and third months after the injection. It was found that neither PRP nor steroid had superior effect on pain and function over three-month period when compared to normal saline group. This study was similar to our study regarding short follow-up period, the patient population and the results. Differently from other groups, significant improvement was observed at eighth week compared to fourth week for grip and pinch measurements in the LR-PRP groups. In the literature, to our knowledge, there is no study comparing the effects PRP and saline injection on grip and pinch measurements in patients with lateral epicondylitis. Along with that, in a study comparing the effect of LR-PRP and corticosteroid on grip measurements in patients with lateral epicondylitis by Gautam et al.,[13] patients were evaluated at the second week, sixth week, third month and sixth month. They found significant improvement in the LR-PRP group at sixth month compared to the pretreatment values. Because of these consequences, we think that the increase in leucocyte concentration in PRP may be a positive effect on tendon healing in the long-term. This may be due to the acceleration of wound healing by increasing local inflammatory response of leukocytes.

Clinical trials evaluating PRP injections in lateral epicondylitis usually do not take into account leukocyte concentration in practice despite increasing number of clinical trials over time. Various studies have reported that leukocytes in PRP have positive antiinfectious, immunoregulatory and angiogenesis effects, and some studies have suggested that leukocytes should be eliminated from PRP.[14] Briefly, the effects of leukocyte concentration in PRP are not fully understood.

In this study, we aimed to investigate the effect of leukocyte concentration in PRP and whether it alters the local inflammatory reaction. For this reason, we questioned the patients for swelling and redness after injection. We failed to detect any difference between groups in terms of pain, function, and local inflammatory response. Riboh et al.[15] examined a number of studies to compare the effect of leukocyte concentration on knee osteoarthritis. They found more side effects in the PRP group than in the other groups. There was no difference between LP-PRP and LR-PRP in terms of efficacy and safety. So, it may be suggested

that the amount of leukocyte does not necessarily affect PRP activity and local inflammation after injection. Further studies are required to assess leukocyte concentration in this issue.

Our results suggest that the PRP treatment is not more effective than placebo in tendon healing at short-term. In a study, Chaudhury et al.[16] injected 3 mL of PRP into six patients who were diagnosed with moderate and severe tendinosis by sonography and evaluated the results on the first and sixth months. In the assessment on the sixth month, authors detected improvement in tendon morphology in five patients when compared to baseline.

This study has some limitations including shorter follow-up period and being a single-center study. As a result, the effect of PRP on tendon repair can alter by time. Therefore, there is a need for long-term studies comparing the effect of LR-PRP and LP-PRP with different preparation techniques. Another limitation of this study is the use of paracetamol because its analgesic effect could lead to bias in the VAS assessment.

In conclusion, lateral epicondylitis does not seem to affected either leukocyte-rich-PRP or leukocyte-poor-PRP on pain and function in the short term. However, further long-term studies with larger sample sizes are needed comparing leukocyte concentrations in lateral epicondylitis.

Declaration of conflicting interests

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