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Ultrasound-Guided Local Injection of Lyophilized Platelet-Rich Plasma versus Autologous Platelet-Rich Plasma in Tennis Elbow

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Abstract:

Objectives: This study aimed to compare the effect of ultrasound-guided injection of lyophilized platelet rich plasma versus autologous platelet rich plasma in the treatment of pain, functional disabilities, and ultrasound changes in the tennis elbow.
Patients and method: This study was carried out on 30 patients with tennis elbow divided into 2 equal groups: group I treated with autologous PRP & group II treated with lyophilized PRP injection at common extensor origin (CEO) ultrasound-guided. All patients were assessed **for pain by visual** analogue scale (VAS), **Functional assessment:** Patient rated tennis elbow evaluation (PRTEE) & Modified Mayo clinic performance index, and **Imaging assessment by musculoskeletal ultrasound**.
Results: Females were (86.66%) of all patients,80% were right-handed, the right side was the most affected side (73.33%) of each group, patients of both groups showed significant improvement of pain (VAS) p= 0.001, function (PRTEE questionnaire with p=0.001 and Mayo clinic performance index p=0.001 and the US changes mainly changes in tendon echogenicity (tendon heterogenicity) p= 0.001 after 6 weeks from injection with significant improvement at group II (lyophilized PRP) in pain (p=0.001) and functional limitation. **Conclusions:** Local injection of PRP is an effective treatment of TE and lyophilized PRP has a better effect than autologous PRP in improving pain, function, and ultrasonographic changes in TE, it can be used in patients with TE who have contraindications to autologous PRP.

Keywords: Tennis elbow, lyophilized & autologous PRP.

Introduction: Tennis elbow (Lateral epicondylitis) is one of the most common overuse syndromes. It is related to excessive wrist extension leading to a degenerative disorder of the common extensor origin CEO at the lateral humeral epicondyle. The

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prevalence ranges from 1% to 3% with a peak prevalence in the fifth decade. [1,2] It is often associated with jobs of manual work and vibrating tools.[3] Pain and tenderness over the lateral side of the elbow that increases with activity (especially resisted wrist extension) and symptoms that improve with rest are the main clinical features.[4] Treatment includes conservative methods such as physical therapy, activity modification, nonsteroidal anti-inflammatory drugs, and local injections of steroids and platelet rich plasma (PRP). [5]

PRP is a concentration of platelets obtained from the blood of the patient and contains growth factors that help to boost the tendon-healing process.[6] For several years, local PRP injection has been used for the treatment of different musculoskeletal diseases. Local PRP injection is a safe, minimally invasive, and effective treatment for improving pain and function by regenerating effects and improving tissue healing of its content of growth factors enhancing cellular chemotaxis, proliferation, and differentiation, removal of tissue debris, angiogenesis, and laying of the extracellular matrix.[7,8]

Lyophilized PRP is a PRP freeze-drying technique for maintaining and stabilizing platelets without serious damage for longer periods (presumably for 6-8 months). The clinical rehydration process then allows the return of membrane and protein components to levels of activity that are strikingly close to those of fresh platelets with documented (counted) high platelet concentrations and growth factors.[9] Platelet-concentrated allogeneic platelets have long been safely administered in the treatment of thrombocytopenia or platelet dysfunction as well as in patients with active platelet-related bleeding or as a prophylaxis in non-cross-matched patients at serious risk of bleeding. In addition to transfusion medicine, a few cases report on oral and maxillofacial surgery treated with allogeneic PRP.[10]

Objectives: This study aimed to compare the effect of ultrasound-guided injection of lyophilized platelet rich plasma versus autologous platelet rich plasma in the treatment of pain, functional disabilities, and ultra-sonographic (US) changes in tennis elbow.

Patients and Method: This study was carried out on 30 patients with symptoms and signs suggesting tennis elbow selected from the outpatient clinic of Physical Medicine, Rheumatology & Rehabilitation Department, XXX University Hospitals.

The ethical approval was obtained from the hospital ethical research committee of the Faculty of Medicine at Xxx University approval code:32768/12/18 and All investigations were explained to all patients, each patient entering the study was informed and gave consent and the trial was conducted according to the Declaration of Helsinki principles.

The patients included in the study were those who suffered from pain on palpation of CEO on the lateral epicondyle and pain elicited from the CEO during resisting wrist

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extension.[11] Patients were aged between 20-60 years old and had a pain duration of 1 to 12 months of lateral elbow pain. The following patients were excluded from the study: patients with arthritis of the elbow such as rheumatoid arthritis, osteoarthritis, seronegative spondyloarthropathies, gouty arthritis, fibromyalgia; patients with previous elbow trauma, infection, or surgery; and patients with referred pain from cervical spine pathology and previous steroid or any local injection (no exclusion of systemic treatment) at the elbow.

All included patients had a full medical history and clinical assessment: general and local of the upper limb; pain assessment (VAS from 0 to 10);[12] and functional assessment (PRTEE evaluates the average pain and function of the affected arm during the previous week). The one-week period offers accurate memory of complain and avoids effects from acute variations in symptoms [13]& modified Mayo clinic performance index that has four parameters: pain, motion, stability, and function.[14]

Lab assessments were done via the exclusion of other rheumatological diseases. Imaging assessment used musculoskeletal ultrasound via a Samsun Medison (UGEO H60) while the patient was supine or seated and the elbow rested on an examination table. A high frequency linear transducer of 12–17 MHz was used to examine the CEO (superficial structure) with enough gel.[15]A full evaluation of the elbow including transverse and longitudinal imaging of all four aspects of the joint [15,16]

The patients were randomly allocated in a non-blinded randomized controlled study via simple random numbers and binned into two groups according to the line of treatment. Group 1 had 15 patients (locally injected by autologous PRP). Blood (20 ml of venous blood) samples were drawn into four vacutainer tubes (5 ml) containing 1 ml of 0.106 M sodium citrate. The samples were gently shaken to mix the anticoagulant with the blood and then centrifuged for 12 mins at 1200 rpm at 22 ^{o}C resulting in the following three layers:

. The lower layer was composed of erythrocytes, the intermediate layer was composed of leukocytes, and the superior layer was plasma.
2. The upper two layers were collected and centrifuged for another 7 mins at 3300 rpm to obtain a two-part plasma: the upper part consisting of platelet-poor plasma and the lower part consisting of PRP.
3. The platelet-poor plasma was first discarded and the tubes were then shaken vigorously for 30 secs to suspend platelets to be ready for injection at the CEO. [17,18]
Approximately 2 mL PRP prepared from autologous blood was US guided injected directly into the area of maximum tenderness, common extensor tendon) using a peppering technique.
Group 2 had 15 patients (locally injected by lyophilized PRP). These patients were treated with US-guided single injection of 2 ml solution of lyophilized PRP vial (2 million platelets in each vial) using a 22G needle into a most tender point (the into maximal point of tenderness at the lateral epicondyle of the elbow joint under complete aseptic technique. The allogeneic GFs was obtained from the Cairo Medical

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Centre Blood Bank (CMCBB). The L-GF vial was prepared from platelets derived from individual whole blood donations. Each unit of platelets was tested for hepatitis B surface antigen, HIV I and II antibodies, HIV p-24 antigen, hepatitis C virus antibodies, and antibodies to *Treponema pallidum* via a licensed assay. Seronegative plasma was further examined by nuclear acid testing. Furthermore, viral inactivation by ultraviolet

radiation and riboflavin were performed by the Mirasol system (pathogen reduction technology system; Terumo BCT, Inc.). The platelets in the buffy coat layer were then activated to release the growth factors. Excess water, cellular elements, and fibrinogen were removed, and the remaining growth factors were ultraconcentrated. The growth factors were then lyophilized. The L-GF vial was supplied as powder in a sealed container. Before use, the vial could reach ambient temperature and was stored between 2 and 8 °C. The L-GF vial content was then mixed with 3 mL sterile water. The vial was gently swirled for 3 minutes and allowed to stand at ambient temperature for 5 minutes to ensure complete protein rehydration.[19]

Just after the injection, every patient was set in a supine position without moving the arm for 15 minutes. Patients were sent home with instructions to limit their use of the arm for about 48 hours and use acetaminophen if the pain is intolerable. The use of non-steroidal drugs was minimized. Follow up was at six weeks via clinical assessment (VAS, PRTEE and Mayo clinic performance index) and US findings.

Statistical analysis used IBM SPSS software package version 20.0 (IBM Corp.). Qualitative data were described using number and percent using Chi-square (X^2) testing. Quantitative data were described using range and mean ± standard deviation using independent sample t-tests. A p-value <0.05 was statistically significant.

Results: Females were (86.66%) of all patients: 80% were right-handed, and the right side was the most affected side (73.33%) in both groups (Table 1). There was a non-significant difference in VAS at activity (p=0.707), Mayo clinic performance index (0.313), and PRTEE (p=0.027) before treatment between both groups. The degree of improvement in VAS at activity, Mayo clinic performance index, and PRTEE was significant in both groups (p = 0.001 more in group II with lyophilized PRP) six weeks from treatment (Table 2).

As regard US changes compared with standard US imaging of normal individual Tendon heterogenicity was the most common US change: It significantly improved after injection in both groups (p=0.001). Focal hypo echogenicity and tendon thickness had no significant change between groups nor before and after treatment. Inter-substance tear was a non-significant abnormality (p=0.143) that improved in both groups with a nonsignificant difference between them (p=0.143) (Table 3).

Enthesophytes (bony irregularities) were the most common bony US changes (80% in group I & 93.3% in group II) with nonsignificant difference between both groups (p=1.0). Tendon calcification was rare (absent in group I & 6.7% in group II). There was a non-significant US change (p=1.0) in both groups (Table 3).

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Discussion: TE may be either due to overuse of the upper limb causing microtears with improper healing resulting in Angio fibroblastic degeneration, inflammatory reaction as a part of a mesenchymal syndrome causing tendonitis, or insufficient blood supply to the affected tendon in which prolonged repetitive micro-trauma of the CEO leads to chronic inflammation that needs a long time to recover.[20] Several treatment options have been proposed to manage the pain and hasten the recovery for patients with lateral epicondylitis.[21]

Injections of platelet rich plasma (PRP) have gained popularity within the sports medicine literature because of their presumed safety and ease of use as a potential treatment for many musculoskeletal problems.[6] Tendons heal more slowly than most other types of tissues partly due to the lower vascular supply.[22] Autologous blood preparations might help with healing because they initiate an inflammatory process while also delivering nutrients and high concentrations of growth factors that may promote tendon healing. [22,23]

So we aimed to compare the effect of ultrasound guided injection of lyophilized platelet rich plasma versus autologous platelet rich plasma in the treatment of tennis elbow

Here, we compared the effect of ultrasound guided injection of lyophilized platelet rich plasma versus autologous platelet rich plasma in the treatment of tennis elbow. There was a statistically significant improvement regarding pain by VAS (at activity), Modified Mayo Clinic Performance Index, and PRTEE questionnaire in autologous and lyophilized PRP between before and six weeks after injection in each group. There was a significant improvement in group II indicating a better effect of lyophilized PRP.

VAS was reported by Varshney et al. (2017) [24] They observed a highly significant improvement of pain score according to VAS after 6 months of autologous PRP injection in tennis elbow patients, which may be due to revascularization and enhancement of healing at the microscopic level. Brkjac et al. (2015) [25] found that the VAS score significantly improves after PRP injection in CEO. Mayo scores were reported by Raeissadat et al. (2014)[26] who showed that the score improved significantly in the autologous PRP group at 4 weeks follow-up and up to 8 weeks. Karaduman et al. (2016) [27] observed that Mayo score improved significantly after autologous PRP injection at one month, two months, and 6 months follow-up periods. Gautam et al. (2015) [28] found that Mayo performance index score improved after 6 months from autologous PRP injection due to biologic regeneration of the tendon as evaluated by US examination. Arirachakaran et al. (2016) [29] found a significant improvement in the PRTEE score during the follow up periods in patients injected with autologous PRP. Palacio et al. (2016) [30] found that PRTEE score improved after 3 and 6 months from autologous PRP injection and Houck et al. (2019) [31] found that PRTEE score improved after autologous PRP injection. This may be due to diffusion

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of blood mediators to the extra and intra-tendinous structures causing regeneration (detected by US).

There was an improvement in tendon echogenicity, tendon thickness, and partial inter-substance tear 6 weeks after PRP injection with nonsignificant differences between both groups. Enthesophytes and tendon calcification showed no changes after 6 weeks from the injection.

This agreed with Ben-Nafa et al. (2018) [32] found that autologous PRP causes gradual progressive improvement in tendon thickness and partial tendon tear 3 months after injection when compared with local injection of steroid and Khattab et al. (2017) [33] who found that there was significant improvement in tendon heterogenicity and partial inter-substance tear 6 months from injecting autologous PRP which may be due to tendon revascularization and regeneration on microscopic level. This contrasts with Shiple (2013) [34] who found that there were limited improvements in pain, Doppler, or tendon thickness 3 months from injection of autologous PRP in TE; they showed a 58% deterioration in the level of improvement 1 month from injection.

Our results agreed with Shukla A et al. (2018) [35] who found that there was no improvement in enthesophyte 6 weeks after autologous PRP injection. Regarding calcification, our results disagreed with Khattab et al. (2017) [33] who found that there was significant improvement in tendon calcification 6 months from injection of PRP—this may be because our patients were followed up for 6 weeks while the improvement of tendon calcification may need a long duration of follow up.

The higher degree of improvement in pain (VAS) and function (Mayo clinic and PRTEE data) of the elbow in group II injected with lyophilized PRP may be explained by higher regeneration power in wounds, e.g., dorsal excisional wound healing in mice within 9 days due to higher concentration of growth factors. [36]

Silva et al. (2018)[37] showed that lyophilization permit higher concentrations and better function of growth factors in lyophilized PRP when cultured in fibroblast and human umbilical vein endothelial cells. Shiga et al. (2016) [38] reported that freeze-dried PRP has higher growth factors with more bone regeneration, trabecular remodeling, and bone strength than fresh PRP when detected histologically. Clinically, lyophilized PRP was used effectively in treating patients with 2nd-degree burns. The healing rate reached 80% within three weeks. [39] Thus, lyophilized PRP is a new type of biological therapeutic recommended for patients with platelet dysfunction syndromes, thrombocytopenia, hyperfibrinogenemia, hemodynamic instability, sepsis, acute and chronic infections, chronic liver disease, anticoagulation therapy, chronic skin diseases, cancer, and metabolic disease. [40]

To the best of our knowledge, until now, there was no report has described lyophilized PRP injected into tennis elbow. Our study showed no apparent local inflammation, allergies, or other adverse reactions in patients treated with lyophilized PRP compared to patients treated with autologous PRP. The lyophilized PRP injection

is simple and easy, and the therapeutic effects and safety are certain. Further studies with longer duration of follow up and larger patient sample are needed for better statistical results.

Conclusions: Local injection of PRP (autologous and lyophilized) is recommended in treating cases of tennis elbow. It is efficient and safe. Lyophilized PRP can be used in patients with contraindications to autologous PRP and has an efficacy comparable to autologous PRP in the treatment of tennis elbow.

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Table (1): Comparison between the two studied groups according to gender and age of the patients.

	Group I (n=15)			Group II (n=15)		Test of sig.	P-value
Gender	**No.**		**%**	**No.**	**%**		
Female	14		93.3	12	80	χ^2= 1.148	**0.283**
Male	1		6.7	3	20		
Age in years Range.	27.0 – 53.0			27.0 – 55.0		t =1.026	**0.276**
Mean ± SD.	34.86 ± 10.487			39.40± 11.87			
Work Nature	Heavy work	11	73.4	10	66.6	χ2= 0.162	0.690
	Non-heavy work	4	26.6	5	33.34		
Handedness Right	12	80		12	80	χ^2= 0.0	1.0
Left	3	20		3	20		
Affected side Right	11	73.3		11	73.3	χ^2= 0.0	1.0
Left	4	26.7		4	26.7		

χ^2: Chi-square
t: Independent-samples t-test

Table (2): Comparison of VAS at activity, Mayo clinic performance index, and PRTEE questionnaire between the two studied groups before treatment & at 6 weeks after treatment.

VAS at activity	Group I	Group II	T. test	P
Before				
Range	5 – 9	5-9	0.139	P1 0.707
Mean ± SD.	7.86± 1.060	8.00± 1.069		
After				
Range	2– 6	1 – 3	5.677	P2 0.001
Mean ± SD.	4.133± 1.060	2.33± 0.617		

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T. test	9.642	17.785		
P value	P3 0.001	P4 0.001		
Before	Mayo clinic performance index			
Range.	55-70	50 – 73	0.978	P1 0.333
Mean ± SD.	63.13± 5.11	60.86± 7.32		
After				
Range.	75– 92	80 – 92	4.529	P2 0.001
Mean ± SD.	81.73± 4.58	88.26± 3.19		
T. test	10.502	13.291		
P value	P3 0.001	P4 0.001		
Before	PRTEE questionnaire			
Range	68-90	75-92	2.379	P1 0.024
Mean ± SD.	79.26± 7.731	85.00± 5.25		
After				
Range	15-40	15 – 30	3.74	P2 0.002
Mean ± SD.	28.86± 7.97	20.06± 4.49		
T. test	17.582	36.412		
P value	P3 0.001	P4 0.001		

P1 comparison between group I and group II (before TTT).,P2 comparison between group I and group II (after TTT), P3 comparison between group I before and after TTT , P4 comparison between group II before and after TTT.

Table (3): comparison between soft tissue (tendon) changes in ultrasound before and after 6 weeks from injection.

	Group I (n=15)		Group II (n=15)		χ^2. test	P
	N	%	N	%		
Tendon heterogenicity						
Before Present	11	73.3%	12	80%	0.189	0.666
Absent	4	26.7%	3	20%		
After Present	0	0.0	0	0.0	0.0	1.0
Absent	15	100%	15	100%		
χ^2.test	17.732		20.012			
P-value	0.001		0.001			
Focal hypoechogenicity						
Before Present	4	26.7%	3	20%	0.189	0.666
Absent	11	73.3%	12	80%		
After Present	2	13.3%	0	0.0	2.142	0.143
Absent	13	86.7%	15	100%		
χ^2.test	0.827		3.328			
P	0.361		0.068			
Tendon thickness						
Before Present	2	13.3%	2	13.3%	0.0	1.0
Absent	13	86.7%	13	86.7%		
After						

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Present	1	6.7%	0	0.0	1.028	0.309
Absent	14	93.3%	15	100%		
χ^2.test	0.371		2.142			
P	0.543		0.143			
Partial inter-substance tear						
Before Present	2	13.3%	0	0.0	2.138	0.143
Absent	13	86.7%	15	100%		
After						
Present	0	0.0	0	0.0	0.0	1.0
Absent	15	100%	15	100%		
χ^2.test	2.138		0.0			
P.	0.143		1.0			
Enthesophyte(bony irregularities)						
Before Present	12	80%	14	93.3%	1.153	0.283
Absent	3	20%	1	6.7%		
After Present	12	80%	14	93.3%	1.153	0.283
Absent	3	20%	1	6.7%		
χ^2.test P value	0.000 1.00	0.000 1.00				
Tendon calcification						
Before Present	0	0.0	1	6.7%	1.028	0.309
Absent	15	100%	14	93.3%		
After Present	0	0.0	1	6.7%	1.028	0.309
Absent	15	100%	14	93.3%		
χ^2.test p. value	0.000 1.00		0.000 1.00			

Fig (1).

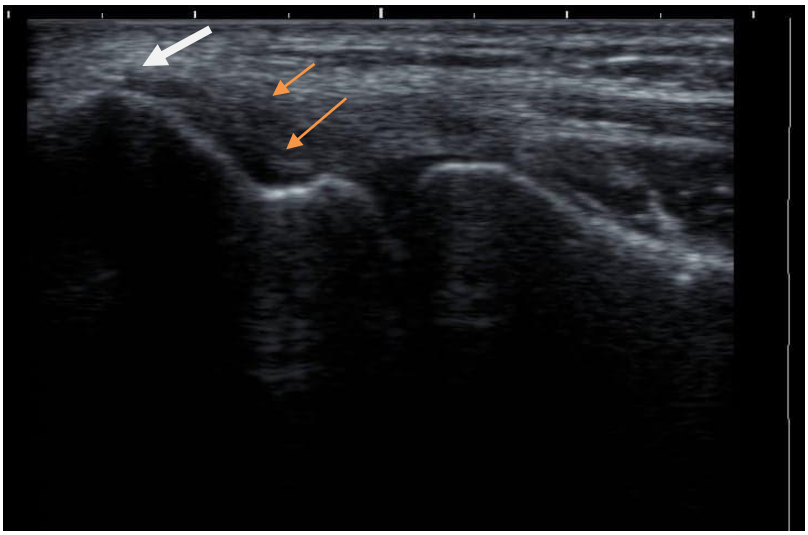


Image (a): Ultrasonographic examination before treatment showed enthesophyte at the lateral humeral epicondyle white arrow) with tendon heterogenicity (orange arrows).

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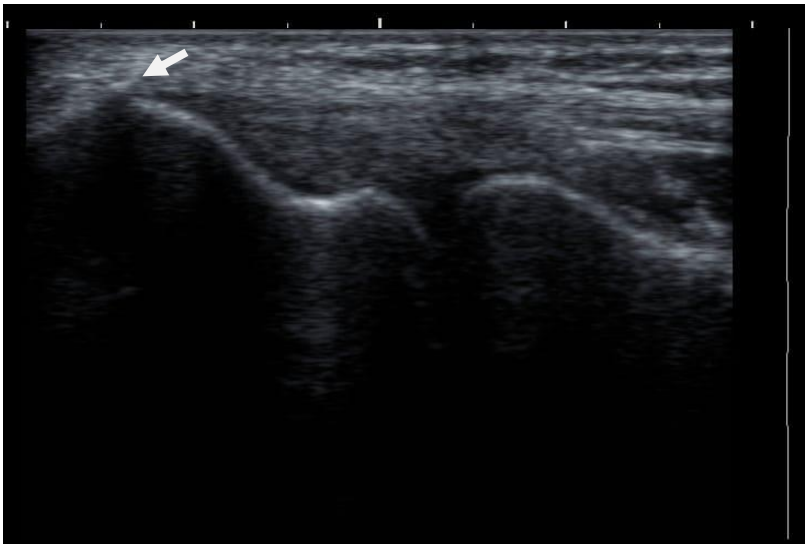


Image (b): Ultrasonographic examination after 6 weeks from autologous PRP injection showed enthesophyte (white arrow) with homogenous tendon (improved heterogenicity).