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Original Article

Decrease of serum biomarker of type II Collagen degradation (Coll2-1) by intra-articular injection of an autologous plasma-rich-platelet in patients with unilateral primary knee osteoarthritis

Rasha Mohamed Fawzy[1], Nashwa Ismail Hashaad[1], Amira Ibrahim Mansour[2]

Abstract

Objective: This study aimed to evaluate the effect of one dose of intra-articular injection of (PRP) in the knee joint on a specific osteoarthritis (OA) serum biomarker of cartilage degeneration, Collagen 2-1 (Coll2-1), over a short period of 3 months. The aim extended to clarify the effect of PRP on the functional status of the osteoarthritic knee joint.

Material and Methods: Sixty patients with primary unilateral knee OA were enrolled in this study. They were subdivided according to Kellgren-Lawrence grading scale (KL) into (Group I): including patients with KL grade < 3 and (Group II): including patients with KL grade ≥3. Patients were asked to complete the Western Ontario and McMaster Universities Arthritis Index (WOMAC) Score. PRP was prepared and injected immediately into the affected knee. Serum Coll2-1 (S.Coll2-1) concentration was measured by enzyme-linked immunosorbent assay (ELISA) kit pre and 3 months after PRP injection.

Results: Significant reduction in S.Coll2-1 concentration in primary knee OA patients; ($p<0.001$) and ($p<0.05$) in group I and group II respectively as well as significant improvements in WOMAC total and WOMAC sub-scores values were noted after single intra-articular PRP injection with maximal functional improvements were achieved after 3 months ($p<0.001$). Mild cases experienced favorable results with no remarkable adverse reactions were observed.

Conclusion: Reduction in specific OA biomarker S.Coll2-1 following intra-articular PRP injection emphasize that PRP could be a promising safe and tolerable effective therapeutic option which improves function from basal states in primary knee OA patients.

Keywords: Platelet-rich plasma, growth factors, serum coll2-1, knee osteoarthritis, Western Ontario, McMaster Universities Arthritis Index Score

Introduction

Osteoarthritis (OA) is a disease of joints, it affects all the intra-articular structures i.e. subchondral bone, synovium, cartilage, menisci and ligaments; resulting in increased metabolism and sclerosis of the subchondral bone, chondrocyte death and extracellular matrix (ECM) catabolism, associated with proliferation of synovial- like endothelial cell, macrophage infiltration and inflammation, which alter the molecular composition of the synovial fluid (1), serum biomarkers of collagen synthesis & degradation and markers of bone & synovium breakdown (2).

These markers particularly, serum collagen 2-1 (S.Coll2-1) level could be used as a predictive factor for response to therapy in OA because, type II collagen is the main protein component and specific for hyaline cartilage (3). It is synthesized as a procollagen molecule which is produced in two forms (IIA) and (IIB). Type IIB procollagen is expressed at high levels in well-differentiated chondrocytes, forming the framework of normal adult cartilage. While type IIA procollagen is temporally expressed in early cartilage (4) and can be re-expressed later by adult articular chondrocytes of affected human osteoarthritic cartilage (5).

The peptide Coll2-1 (108HRGYPGLDG116) and its nitrated form, Coll2-1NO2 (108HRGY[NO2] PGLDG116), are located in the triple helix of the type II collagen molecule and are 2 of the most recently used biomarkers of collagen degradation (6).

Knee joint is considered among the most frequently joints affected by OA (7). Treatment of chondral disease is difficult as the regeneration capability of articular cartilage is restricted due to its lack of vessels and

[1] Department of Rheumatology, Rehabilitation & Physical Medicine, Benha University School of Medicine, Benha, Egypt

[2] Department of Clinical and Chemical Pathology, Benha University School of Medicine, Benha, Egypt

Address for Correspondence: Rasha Mohamed Fawzy, Department of Rheumatology, Rehabilitation & Physical Medicine, Benha University School of Medicine, Benha, Egypt

E-mail: dr.rasha.fawzy@hotmail.com

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nerves. Various pharmaceutical approaches, have been widely used though, none postpone the progression of the disease or stop joint damage. In addition, to increasing the adverse effects of these drugs which increases with age (1).

Platelet- Rich- Plasma (PRP) is 1,000,000 per µL platelet count, which is five times the normal concentration found in whole blood. Using (PRP) in clinical and basic practical research is rapidly increasing. Experimental data supporting the valuable therapeutic effects of PRP in soft tissue healing, ligament and bone regeneration, also in modulation of angiogenesis and decreasing inflammation, with subsequent pain relief, improvement of function and probably substitute the chondrodestructive environment with elevated levels of anabolic and chondroprotective cytokines which stimulate cartilage regeneration at the site of injury (8).

Aim of the work

This study aimed to evaluate the effect of one dose of intra-articular injection of PRP in the affected knee joint on a specific OA serum biomarker, Coll2-1, to find out the effect of this therapy on cartilage degeneration over a short term period of 3 months. The aim extended to clarify the effect of PRP on the functional status of the osteoarthritic knee joint.

Material and Methods

Study approval

The study was approved by Research Ethics Committee, School of Medicine, Benha University, Egypt. The aim, methods of PRP and adverse effects of the study was explained to all participants, and an informed written consent was obtained from all of them prior to participation in this study.

Subjects

Sixty patients with unilateral primary knee OA diagnosed according to the American College of Rheumatology (ACR) classification criteria of knee OA (9) were enrolled into this study. They were divided into two groups according to the Kellgren and Lawrence (KL) scale (10): (Group I): including patients with < grade 3 knee OA and (Group II): including patients with ≥ grade 3 knee OA.

All patients reported a history of chronic pain (at least 4 months) or swelling.

These patients were recruited from the Rheumatology, Rehabilitation and Physical medicine outpatients' clinic and inpatients' department of Benha University Hospitals between April 2015 and October 2015.

Exclusion criteria included

Cases with bilateral symptomatic knee OA; age older than 70 years; receiving physical therapy, intra-articular injections of steroid, or hyaluronic acid in the last 6 months before PRP injection; secondary OA [past history of fracture knee bones, meniscal lesion, cruciate ligament lesion or major deformity (varus more than 5^0, valgus more than 5^0)], Systemic disorders (diabetes, rheumatoid arthritis, hematological diseases (coagulopathies), severe cardiovascular diseases or malignancy), Infectious disorders (septic arthritis, viral arthritis, fungal arthritis and signs of local inflammation), Immunosuppression or patients on therapy with anticoagulants-anti-aggregants and use of nonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided 2 weeks before and at least 2 weeks after the procedure to avoid inhibition of the effects of growth factors and the healing ability.

Methods

All patients were subjected to full history taking including history of medication use, thorough clinical examination and complete knee joint examination. Patients were asked to complete the Western Ontario and McMaster Universities Arthritis Index (WOMAC) osteoarthritis index questionnaire (11). In our study, the patients' WOMAC sub-scores (pain, stiffness, function) and total WOMAC score were calculated.

Kellgren and Lawrence (10) grading was obtained based on standard weight-bearing anteroposterior and lateral radiographs of the affected knee.

About 35mL of venous blood was collected from each study subject by sterile venipuncture, about 3 mL allowed to clot naturally for 30 minutes, then centrifuged at 1000xg for 15 minutes, sera were separated and kept frozen at -80°C till used for ELIZA. The remaining about 30 mL of venous blood samples were collected in sterile sodium citrated tubes. The tubes were centrifuged at 1800 rpm for 15 min to separate erythrocytes, then at 3500 rpm for 10 min to concentrate the platelets (12). By this method, 5 mL of PRP-were obtained. It has been noted that using freshly-harvested PRP may preserve all the platelet functions better. PRP was injected immediately without storage in the affected knee joint with subjects in a supine position and the knee joint in full extension using a common lateral approach. Then patients were asked to flex and extend their knees actively a few times to spread the PRP throughout the joint.

Laboratory procedures included the following:

- Complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), coagulation profile.
- Measurement of Serum Coll2-1 (S.Coll2-1):

Serum Coll2-1was measured using a commercially available ELISA kit (COLL2-1 Serum ELISA KIT; artialis S.A. Liège, Belgium) according to the manufacturer's protocol (13). The assay detects a degradation fragment containing a 9 amino acid sequence (HRGYPGLDG), named Coll2-1, is derived from type II collagen and is released into the synovial fluid following the action of collagenases and gelatinase B.

In principle, it is a competitive immunoassay utilizing a synthetic peptide pre-coated onto the ELISA plate for the quantification of the corresponding antigen in serum samples. A binding competition between the immobilized peptide and the peptide contained in the standards or samples takes place upon addition of the antibody Ab-Coll2-1. After removal of the unbound peptide, a peroxidase-conjugated goat anti-rabbit antibody is added into each well to detect and quantify the level of competitive binding. After washing of the unbound detection antibody, the antibody-antigen complex is detected by a chromogenic reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid solution and the absorbance was read at 450 nm within 30 min. The range of the assay is 31.25 nM - 2000 nM with a sensitivity of 26.9 nM.

Follow up assessment: re-evaluation was done 1 week (search for any adverse reactions), and patients were asked to complete WOMAC score at 1 month and 3 months after PRP injection with S.Coll2-1 concentration was re-measured for all the patients after 3 months from PRP injection.

Statistical analysis

The statistical analysis was conducted using Statistical Package of Social Sciences (SPSS) 16.0 for Windows (SPSS Inc.; Chicago, IL, USA).The collected data were summarized in terms of mean±standard deviation (SD) for quantitative data and frequency and percentage for qualitative data. In the statistical comparison between the different groups, post-hoc test was used to detect significance difference in-between groups. P value $<0.05^*$ was considered statistically significant and p value $\leq 0.001^{**}$ was considered highly significant.

Table 1. Characteristics of the studied osteoarthritis patients

Variable	Group I (KL grade < 3) N: 35 (58.5%)	Group II (KL grade ≥3) N: 25 (41.5%)	p
Age (years)	62.5±3.23	67.8±1.5	>0.15
Female\Male	30\5	18\7	>0.32
BMI in kg/m2 (X±SD)	22.27±1.16	21.79±2.34	>0.8
Disease duration (years)(Mean±SD)	3.5±1.5	5.9±6.4	<0.014*
S.Coll2-1 (nM) at base line visit (X±SD)	184.69±38.59	220.56±63.13	<0.032*

N: number; BMI: Body Mass Index; S.Coll2-1: Serum Coll2-1; KL: Kellgren-Lawrence grading scale; Significant p<0.05*, insignificant p>0.05

Table 2. Comparison between mean serum Coll2-1 level at base line and 3 months after PRP injection in patients with unilateral primary knee osteoarthritis

Group	Follow ups	S.Coll2-1 (nM)	p
Group I (N:35) (KL grade < 3)	Base line	184.69±38.59	0.0005
	3 months	102.07±8.54	
Group II (N:25) (KL grade ≥3)	Base line	220.56±63.13	<0.034*
	3 months	189.47±28.34	

N: number; S.Coll2-1: serum Coll2-1, KL: Kellgren-Lawrence grading scale *Significant p<0.05, **Highly significant p≤0.001

Table 3. Comparison between WOMAC index variables at base line and follow up visits in patients with unilateral primary knee osteoarthritis

Group	Follow ups	WOMAC Total Mean±SD	WOMAC Pain Mean±SD	WOMAC Stiffness Mean±SD	WOMAC Function Mean±SD
Group I (KL grade < 3)	Base line	70.8±1.5	14.3±0.2	3.1±0.1	53.4±1.2
	1m	62.7±2.6	12.1±0.4	2.6±1.3	48.0±0.9
	3m	48.4±1.4	8.1±0.6	1.2±0.5	39.1±0.3
p		0.0004	0.0003	0.0002	0.0004
Group II (KL grade ≥3)	Base line	84.9±1.9	17.3±0.4	5.5±0.2	62.1±1.3
	1m	78.8±0.7	15.9±0.2	4.7±0.1	59.4±0.4
	3m	72.7±2.1	13.2±0.3	3.6±0.2	53.5±1.6
p		0.0004	0.0003	0.0002	0.0004

WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; KL: Kellgren-Lawrence grading scale **Highly significant p≤ 0.001

Results

Table (1): Characteristics of the studied osteoarthritis patients:

As regard age, sex distribution and BMI there were no statistically significant difference among the studied groups (p>0.05). The mean disease duration in group I was 3.5±1.5 years and in group II was 5.9±6.4 years with a statistically significant difference (p<0.05*).

As regard mean S.Coll2-1 level at base line visit; there was a statistically significant difference (p<0.05*) being higher in group I than group II.

Table (2): Comparison between mean serum Coll2-1 level at base line and 3 months after PRP injection in patients with unilateral primary knee osteoarthritis:

As regard the mean S.Coll2-1 level; there was a statistically highly significant decrease (p<0.001**) when comparing their serum levels at the base line visit and after 3 months from PRP injection (184.69±38.59 nM versus 102.07±8.54 nM in group I respectively and a statistically significant decrease (p<0.05*) in group II (220.56±63.13 nM versus 189.47±28.34 nM respectively).

Table (3): Comparison between WOMAC index variables at base line and follow up visits in patients with unilateral primary knee osteoarthritis.

In group I and group II; there were a statistically highly significant difference as regard WOMAC total and WOMAC sub-scores values (p<0.001**) when comparing their base line values and after 3 months from PRP injection.

In group I the mean±SD of WOMAC Total, WOMAC Pain, WOMAC Stiffness, WOMAC Function were 70.8±1.5, 14.3±0.2, 3.1±0.1 and 53.4±1.2 respectively and decreased after 1 month follow up to be 62.7±2.6, 12.1±0.4, 2.6±1.3 and 48.0±0.9 respectively with significant improvements were achieved after 3 months from PRP injection where their values changed to 48.4±1.4, 8.1±0.6, 1.2±0.5 and 39.1±0.3 respectively.

In group II the mean±SD of WOMAC Total, WOMAC Pain, WOMAC Stiffness, WOMAC Function were 84.9±1.9, 17.3±0.4, 5.5±0.2 and 62.1±1.3 respectively and decreased after 1 month follow up to be 78.8±0.7, 15.9±0.2, 4.7±0.1 and 59.4±0.4 respectively with significant improvements were achieved after 3 months from PRP injection where their values changed to 72.7±2.1, 13.2±0.3, 3.6±0.2 and 53.5±1.6 respectively.

In this study we observed synovitis after PRP injection in four patients, eight patients complained of temporary pain following the injection and resolved within three days.

Discussion

Osteoarthritis (OA) is thought to be a main cause of knee pain and locomotor system disability (14). Medications which protect or stimulate healing of the cartilage are still under investigations. These include medications which inhibit cytokine production, gene therapy, artificial chondrocytes, and growth factor (GF) that enhances the production of chondrocyte matrix, stimulates proliferation of chondrogenic cells and decreases nuclear factor kappa B activation (15, 16).

Type II collagen is the main protein in cartilage, forming up to 50% of its extracellular matrix. This study showed that there was a statistically significant difference (p<0.05) as regard mean S.Coll2-1 levels being higher in group II than group I. This was consistent with Henrotin et al. (3) who reported that the serum concentrations of Coll2-1 and Coll2-1 NO2 were significantly higher (P<0.05) in knee OA patients with KL III/IV compared to KL I/II patients also; this was in agreement with Denis et al. (17). Meanwhile, this was in great discrepancy with Deberg et al. (13) who found no relationship between the levels of Coll 2-1 and Coll 2-1 NO2 in the serum and the radiological OA severity.

Different PRP regimens were used as trials for treatment of knee OA; we used single injection and noted that PRP application reduced pain and improved clinical outcomes. Better results were achieved in group I than group II with maximal improvement after 3months from PRP injections. These results were similar to those of Kwon et al. (18) who observed that PRP was effective in all stages of degeneration; with greater regenerative results in mid- or mild-mid level OA. Noteworthy, Halpern et al. (19), Jang et al. (20) and Pourcho et al. (21) examined the effectiveness of a single dose of PRP in OA patients with KL grade (0-II) early knee OA; pain, functional and clinical scores significantly decreased. Although Halpern et al. (19) reported improvements at the beginning, functional and clinical scores started to deteriorate at six months and one year from baseline.

Jang et al. (20) also reported that older ages, associated with patellofemoral degeneration resulting in decreased the probability for PRP injection therapy and symptoms tended to become worst one year after the injection.

Furthermore, Çalış et al. (22) studied the effect of PRP therapy in patients with grade 3-4 (advanced) knee OA and observed short and long term clinical, functional using (visual analog scale, WOMAC index, six-minute walk test) and radiological improvements in their patients compared to before treatment with subsequent improvements of their quality of life which support the beneficial regenerative effect of PRP injections even in advanced OA patients.

Kon et al. (23) supported the hypothesis that, additional biological mechanisms were responsible for the improvement of OA symptoms following PRP therapy. In advanced OA stages, PRP may not affect the anabolic process of chondrocyte directly, but it regulates joint homeostasis and the cytokine level thus it has an anti-inflammatory effect (24).

Other investigators used more than single PRP injection in treatment of knee OA. Kavadar et al. (25) found that 3 injections separated by 2-week intervals to be more effective for the improvement of pain and mobility than 2 injections in Grade 3 OA patients; however, no significant differences were observed in the WOMAC values. A significant effect was observed in the early period after a single injection of PRP, but the effect decreased in a short time.

Different methods were used to prove the efficacy of PRP as a treatment modality that alter cartilage degradation; in our study, we assess the mean S.Coll2-1 level as a specific marker of cartilage degradation and we found a statistically significant reduction in the mean S.Coll2-1 level at baseline compared to results obtained after 3 months from PRP injection.

In Çalış et al. (22) study they used musculoskeletal ultrasound (MSUS) to assess cartilage thickness and demonstrated significant increase in the thicknesses of articular cartilage after treatment at third and sixth months compared to before treatment ($p<0.05$). These results were similar to those of Hassan et al. (26) who reported a significant improvement in doppler activity ($p=0.04$) and synovial thickening ($p< 0.001$) after 6 months of PRP injection. Although Halpern et al. (17) used qualitative magnetic resonance imaging and didn't report any change per compartment in at least 73% of patients at one year.

An old case report has been described, where plasma rich in growth factors was used to treat an articular cartilage avulsion in a soccer player with complete articular cartilage healing (27). Regarding animal studies, Wu et al. (28) investigated the feasibility of PRP to support chondrogenesis; they reported that gelled PRP was successfully used to deliver chondrocytes in cartilage defects in a rabbit model.

Kon et al. (23), Filardo et al. (29), Hassan et al. (26) and Jang et al. (20) studies, reported only postinjection swelling and pain with no major adverse events were observed in the PRP treated group. The cause of mild knee pain and swelling may be related to a mild inflammation related to the PRP injection, and distension of the synovial joint by PRP fluid. Patel and colleagues reported other mild complications such as nausea and dizziness, which were of short duration (30).

Reduction in specific OA biomarker S.Coll2-1 following intra-articular PRP injection suggest that PRP could be used as a promising safe, tolerable and effective therapeutic option which improves function from basal states in primary knee OA patients.

Key messages: PRP could be used as a safe treatment for both early and late OA cases with better results obtained in early cases.

Ethics Committee Approval: Ethics committee approval was received for this study from Benha University School of Medicine, Egypt.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - R.M.F., N.I.H., A.I.M.; Design - R.M.F., N.I.H.; Supervision - R.M.F., N.I.H.; Resources - R.M.F., N.I.A., A.I.M.; Materials - R.M.F., N.I.H., A.I.M.; Data Collection and/or Processing - R.M.F., N.I.H., A.I.M.; Analysis and/or Interpretation - R.M.F. N.I.H.; Literature Search - R.M.F., N.I.H., A.I.M.; Writing Manuscript - R.M.F., N.I.H., A.I.M.; Critical Review - R.M.F.

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