RESEARCH ARTICLE

Effectiveness of Platelet-Rich Plasma Based on Gene Expression in Knee Osteoarthritis

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Abstract

Background: Intra-articular injection of Platelet-rich plasma (PRP) is an effective method for the treatment of patients with knee osteoarthritis (OA). This study aimed to assess the effects of PRP injection on OA, based on gene expression analysis.

Methods: A sample of 30 subjects with knee OA was asked to complete the Persian versions of the Intermittent and Constant Osteoarthritis Pain (ICOAP) and Knee and Osteoarthritis Outcome Score (KOOS). Thereafter, the expression of *IGF-1*, *HIF-1*, cartilage oligometric matrix protein (*COMP*), and bone morphogenetic proteins (*BMP2*) were compared in the patient before and 1 month after PRP injection using real-time polymerase chain reaction (PCR).

Results: According to the results of the study, the expression of *IGF-1*, *HIF-1*, *COMP* and *BMP2* were reported to be higher in subjects with PRP injection; however, only the up-regulation of *IGF-1* was statistically significant ($P<007$). Moreover, the significant change in the KOOS and ICOAP scores was attributed to PRP injection ($P<0.01$).

Conclusion: Intra-articular injections of PRP were reported to ease the pain, decrease the stiffness, and improve quality of life in patients with knee OA through the promotion of *IGF-1* expression.

Level of evidence: V

Keywords: IGF-1, Osteoarthritis, Platelet-rich plasma

Introduction

Osteoarthritis (OA) is a progressive disease characterized by the degeneration of articular cartilage and alteration in joint tissues accompanied by other various symptoms, such as pain, stiffness and disability. The OA as a frequent form of joint disease and fourth leading cause of disability exerts a great impact on the quality of life by inflicting a lot of pain on patients. In OA, the cartilage is degenerated, and due to the lack of blood vessels in cartilage, regeneration cannot be performed effectively (1, 2). The major risk factors for OA include age, gender, body mass index (BMI), joint injury, and life style (3).

Although the pathogenesis of this disease is complex and not well understood, OA is associated with an imbalance of anabolic and catabolic activities in the cartilage resulting in cartilage loss (4). Knee arthritis is one of the most common form of arthritis which affects all races, genders and ages groups. To make the matters worse, despite significant therapeutic advances, there is no definite treatment for OA (5). The currently used methods of treatment just ease the pain; however, advanced OA usually requires surgery procedures. Regarding this, the introduction of new therapeutic approach is of utmost importance, due to OA incidence increase (6).

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The application of platelet-rich plasma (PRP) as a newly recognized therapeutic treatment for OA (7-8). PRP is a plasma fraction from autologous blood with platelet concentration above the baseline (9). Platelets contain a variety of growth factors (GFs) and many important bioactive proteins and anti-inflammatory cytokines which mediate cartilage healing (10). Different types of GFs are released from platelets, including platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor-β, basic fibroblast growth factors, insulin like growth factor (IGF), epidermal growth factors, and many other factors (11, 12).

In vitro researches on the effect of PRP on chondrocyte revealed the strong positive effect of PRP chondrocyte proliferation and the inflammatory and cell differential properties of PRP (13, 14). Therefore, PRP is revealed to play a major role in cartilage regeneration. The present study aimed to analyze the efficacy of the intra-articular injection of PRP in patients with knee OA according to multidimensional pain questionnaires and gene expression analysis, before and 1 month after the intra-articular injection of PRP.

Materials and Methods
Platelet-rich plasma preparation and injection
The PRP was obtained from blood samples collected from 30 patients afflicted with knee OA. A total of 35 mL of blood was collected under aseptic conditions to produce PRP according to manufacturer's protocol (Rooyagen Kit, Arya Mabna Tashkis Corporation, RN: 312569). Autologous PRP was isolated separately for each patient. The Intra-articular Knee Injection (8 mL PRP) was administered using 22G needle without any local anesthetic agent.

The patients were evaluated before and 1 month after the injection, using questionnaires of Knee injury and osteoarthritis outcome scores (KOOS) and the intermittent and constant pain score (ICOAP). All questionnaires were filed out by a physical medicine and rehabilitation specialist.

RNA, reverse transcription polymerase chain reaction, and Real-Time polymerase chain reaction
The RNA was collected from patient's blood before PRP injection and 1 month later, using the RNeasy blood mini kit (Qiagen, Germany) according to the manufacturer's protocol. The quality and quantity of RNA was analyzed by optical density measurements of 260/280nm ratio and gel electrophoresis. Complementary DNA (cDNA) was prepared from purified RNA for each sample utilizing the cDNA synthesis kit (fermentas, Germany). The relative expressions of responsible genes for cartilage synthesis were determined using Real-Time PCR approach. Primer specifications are shown in Table 1. To this end, 1μg synthesized cDNA was mixed with 2x SYBR® Green Master Mix (Genetbio, South Korea) and the mixture was subjected to the Corbet 6000 Real-Time PCR Detection System (Qiagen, Germany). Amplification conditions were as follows: 10 min at 95 ºC, 40 cycles of 30 s at 95 ºC, 30 s at 60 ºC and 30 s at 72 ºC. The data were normalized to GAPDH gene expression, and relative expression was then calculated using $2^{-\Delta\Delta Ct}$formula. Gene expression experiments were performed in triplicate.

Statistical analysis
Statistical analyses were performed in SPSS software version 18 using t-test and one-way ANOVA test. The obtained results are presented as mean±SD. A P-value less than 0.05 was considered as statistically significant

Results
Out of 30 participants who were candidates for intra-articular PRP injection, 63.3% (n=19) of the cases were female. The mean age of subjects was 58.08±14.15 years (age range: 29 -73 years) and the mean of BMI was 30.21±4.657 kg/m². All patients were reported to

Table 1. List of primers used in current study IGF1: Insulin-like growth factor 1, BMP2: Bone Morphogenetic Proteins, HIF-1a: Hypoxia inducible factor, COMP: Cartilage oligometric matrix protein, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase			
Name of Gene	sequence	Accession number	Length of products
IGF1	F: TCTACCTGGCACTCTGCTTG R: GGTCCACACACGAACTGAAG	NM_001111285.2	106 bp
BMP2	F: TCAAGCCAAACACAAACAGC R: CCACGATCCAGTCATTCCA	XM_011529323.2	101 bp
HIF1a	F: CGATGACACGGAAACTGAAG R: CAGATTCAGGTAATGGAGACA	NM_001243084.1	125 bp
COMP	F: AACTCAGGGCAGGAGGATGT R: TGTCCTTTTGGTCGTCGTTC	NM_000095.2	196 bp
GAPDH	F: AGGGCTGCTTTTAACTCTGGT R: CCCCACTTGATTTTGGAGGGA	NM_001289746.1	206 bp

show significant improvement in their KOOS and ICOAP scores 1 month after injections, compared to the scores obtained before injection [*P<0.05*; Table 2]. The patients with knee arthritis showed a noticeable improvement in different subscales of KOOS. The scores of the subscales of symptoms, pain, and function in daily living were reported as 67.86±1.01, 62.99±5.162 and 59.32±12.89, respectively. The results of ICOAP are presented in table 2. In this questionnaire, the mean scores in the PRP group revealed a much improved result of 51.67±9.2 at the end of the study, compared to the value of 85.86±2.5 at the baseline.

Real-Time PCR was utilized to determine the expression of responsible genes in the cartilage synthesis, before PRP injection and a months later. The expressions of *IGF-1*, *HIF-1*, *COMP,* and *BMP2* in relation to *GAPDH* are displayed in Figure 1. As shown in this figure, significant differences were observed for *IGF-1* before and after PRP injection using Real-Time PCR. The average expression of *IGF-1*, *HIF-1*, *COMP* and *BMP2* was reported to be markedly higher a month after PRP injection (101.59±11.03, 53.3±8.14, 8.46±1.02, 24.36 ±4.48 folds, respectively). However, only the up-regulation of *IGF-1* was found to be statistically

Table 2. Results of knee injury and Osteoarthritis Outcome Score y and ICOAP at the pre- and post-treatment stages				
	Variables	**Baseline (±SD)**	**Week 4 (±SD)**	**Adjusted *P-value***
KOOS	Pain	34.72±4.158	67.86±1.01	0.0010
	Symptoms	15.12±3.6	62.99±5.162	< 0.0001
	ADL	16.1±2.38	59.32±12.89	< 0.0001
	Sport/Rec	10±3.015	23.33±3.33	0.6470
	QQL	62.5±2.5	22.92±7.5	0.3691
ICOAP		85.86±2.5	51.67±9.2	0.001

Knee injury and Osteoarthritis Outcome Score consists of five subscales; pain, other symptoms, function in daily living (ADL), function in sport and recreation (Sport/Rec) and knee related quality of life (QOL). A normalized score (range 0 to 100 with 0 indicating extreme problems and 100 indicating no problem) is calculated for each subscale. In ICOAP index score 100 means no problem and 0 mean extreme problem.
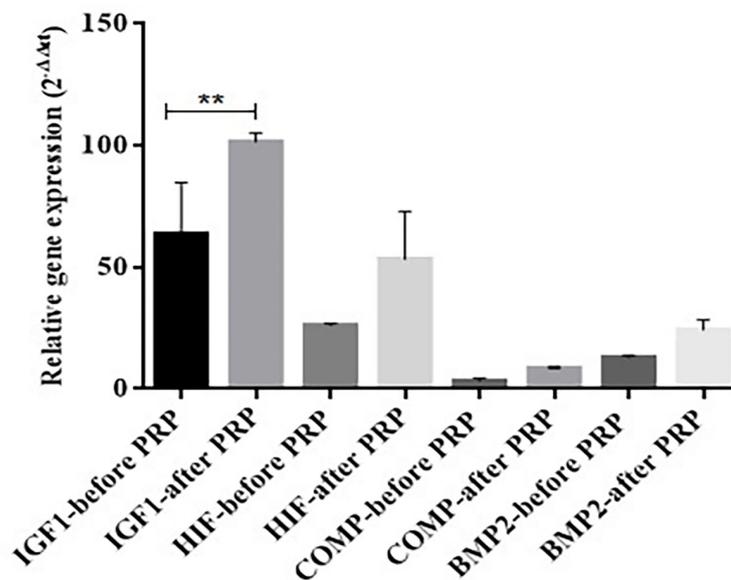

Figure 1. Gene expression analysis before and one month after Platelet-rich plasma injection in knee osteoarthritis patients. (The IGF-1 expression significantly increased one month after injection (P<0.05). The comparison of the expression of gene between subjected with and without osteoarthritis indicated that the expression of all genes [i.e., IGF-1, HIF, COMP, and BMP2] up-regulated even before PRP injection in comparession to that in intact individuals).
IGF1: Insulin-like growth factor 1, HIF-1: Hypoxia inducible factor, COMP: Cartilage oligometric matrix protein, BMP2: Bone Morphogenetic Proteins.

significant ($P<0.05$). We expected that based on our hypothesis, the expression of all genes be meaningfully up-regulated in all patients one month after PRP injections.

Discussion

The PRP offers a rich source of autologous growth factor concentration that accelerates the healing process. In the present study, PRP was applied for patients afflicted with knee OA, and the healing effects were evaluated using gene expression analysis for the first time. The results of the study revealed a significant improvement in most of the reported scores for KOOS and all of ICOAP scores. Therefore, PRP can be regarded a valuable treatment for relieving the great pain of the patients afflicted with OA.

The PRP is a complex of growth factors, and numerous studies demonstrated the effectiveness of growth factors in chondrogenesis and prevention of joint degeneration (15, 16). Maleki-Fischbach et al. reported that both genders are comparable regarding OA affliction until the age of 50 years. However, the prevalence of this disease was reported to increase significantly in women after this age, due to unknown reasons (17). However, no significant difference was observed between females and males in this respect in the present study.

Based on the results of the previous studies, PRP should be considered an effective treatment for knee OA (7-8). In a study on the comparison of different numbers of PRP applications in 102 knee OA cases, Kavadar et al. evaluated the efficiency of treatment using Western Ontario and McMaster Universities Arthritis Index, Visual Analog Scale (VAS), and Timed-up and Go test at the beginning of treatment, as well as 1, 3 and 6 months later (18). They indicated that the application of at least two injections of PRP is an effective procedure in the treatment of knee OA.

Conversely, the results of another study conducted by Patel et al. entitled "The comparison of the effects of one and two injections of PRP with normal saline injections in knee OA patients" revealed that although PRP was effective in improvement of patient's symptoms, effectiveness of a single injection of PRP was more than two injections (19). In the current study, we indicated an improvement in pain based on KOOS and ICOAP questionnaires after an injection of PRP. These observed differences in various studies may be due to the utilization of PRP purification methods which are crucial for the experimental success.

The results of numerous studies revealed the effectiveness of PRP in the alleviation of pain symptoms and improvement of knee scores (7-8). Hart et al. reported no reduction in cartilage thickness based on magnetic resonance imaging (MRI) performed on the patients with knee arthritis who received six manually prepared PRP injections at weekly intervals (20). Dhollander et al. treated five patients with knee pain using a single injection of PRP. In the mentioned study, MRI scans were indicative of degenerative chondral lesions.

The authors noted substantial clinical improvement in VAS and KOOS scores at the baseline and 12 and 24 months after the intervention; however, no response was observed in the analysis of cartilage in MRI (21). In a prospective study conducted by Gobbi et al., patients with a diagnosis of degenerative cartilage lesions were treated with a series of two injections of PRP. The 50 patients were clinically evaluated before and at the end of the treatment and during the follow-up using appropriate questionnaires. They showed noticeable improvements in all clinical scores, including improved joint function and decreased pain (22).

Moreover, IGF-1 plays a crucial role in the up-regulation of cartilage matrix biosynthesis. The enhanced expression of this factor in OA represent the effort of cartilage in regeneration, although it is not the only effective factor (23). The primary sign of pathology in OA is cartilage matrix degradation, and the expression of IGF-1 induces the synthesis of matrix (24). In conclusion, IGF-1 had a potential to repair and regenerate cartilage defects. Additionally, Hochberg et al. reported that the serum levels of IGF-1 were reduced in OA patients (25). Bone Morphogenetic Proteins (BMP2) were also revealed to promote the synthesis of cartilage matrix and induce Matrix metalloprotease-13 (MMP-13) expression for modulation chondrocyte differentiation (26).

The role of BMP2 can vary in different patients. For instance, BMP2 receptors were revealed to play a key role in the chondrocytes of OA patients and account for the inability of BMP2 in chondrogenic differentiation. Cartilage oligometric matrix protein (COMP) acts as a biological marker for cartilage degradation (27). The results of a meta-analysis performed by Hoch in 2011 indicated that serum COMP was consistently elevated in knee OA patients (27). Serum COMP level was determined as an OA biomarker (28). The COMP is a non-collagenous glycoprotein that binds to collagen for stabilization the network of articular cartilage collagen fiber and is released as bound proteins are cleaved from the cartilage matrix in OA (29). However, the relationship between COMP expression and pain is still unknown.

Furthermore, hypoxia inducible factor (HIF-1) is considered a regulator of response to hypoxia. Cartilage is a hypoxic tissue in which HIF-1 is responsible for the survival and growth arrest of chondrocytes during cartilage development (30). Yodoh et al. indicated that the expression of HIF-1 increased in degenerated cartilage, compared to that in intact cartilage (31). The OA is an active process and a network of mechanisms reacting to mechanical stress or joint injury. The HIF-1 expression significantly increased in chondrocytes by mechanical stress. Therefore, the results of the current research and similar studies were indicative of PRP effectiveness as a treatment for OA patients and revealed the possible therapeutic effect of growth factors in the treatment of the disease.

The main important limitation of the current research included the absence of control group, small sample size, and short term follow-up. Although in the present

research analyzed the effectiveness of one series of PRP injection in gene expression in OA patients, further studies are required with larger sample sizes, longer follow-up, and analysis of more responsible genes in cartilage regeneration.

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