Assessment of Therapeutic Effects of Platelet-Rich Plasma in Knee Osteoarthritis: Possible Role of Inflammatory Cytokines


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Abstract: Osteoarthritis (OA) is a multifactorial disease that commonly affects the knee. Tumor Necrosis Factor-α (TNF-α) regulates inflammation during OA. Macrophage Migration Inhibitory Factor (MIF) may be involved in the pathophysiology of arthritis. Platelet-Rich Plasma (PRP) may reduce pain associated with OA. The present study assessed the possible therapeutic effects of PRP in patients with OA of the knee with varying severities. A prospective study was performed in 90 patients diagnosed with mild (30 cases), moderate (30 cases) and severe (30 cases) knee OA. Three Intra-Articular (IA) injections of PRP were administrated, 2 weeks apart, were received. Pain score and MRI Osteoarthritis Knee Score (MOAKS) were assessed. Serial synovial fluid cytokine assays to measure Tumor Necrosis Factor-α (TNF-α) and macrophage Migration Inhibitory Factor (MIF) were performed using commercially available ELISA kits. The assays were performed pre-injection (S1), 2 weeks after the first IA injection (S2) and 2 weeks after the second IA injection (S3) for all patients. The mean values of pain score and synovial TNF-α and MIF levels were significantly higher (S1, pre-injection) among severe OA than among those with either mild or moderate cases, p˂0.05 for all. Pain score and synovial TNF-α and MIF levels at S3 were significantly lower in those with mild, moderate and severe OA than the corresponding S1 values, (p˂0.05 for all). There was significant improvement in synovitis in both mild and moderate cases, (p<0.05 for both). The IA injection of PRP reduces synovial fluid TNF-α and MIF levels significantly and exhibit significant therapeutic effects on synovitis by reducing inflammatory cytokine levels and bone marrow lesions primarily for mild knee OA and to a lesser extent for moderate cases.

Keywords: Platelet Rich Plasma, Knee Osteoarthritis, MRI Osteoarthritis Knee Score, Pain Score, Synovial Fluid, Tumor Necrosis Factor-α, Macrophage Migration Inhibitory Factor

Introduction

Osteoarthritis (OA) is a disease primarily affects the knee. It is defined as a progressive loss of joint function and pain from the gradual deterioration of the articular cartilage (Kennedy et al., 2018). Unfortunately, it is a common malady because of the frequent use and stress of the knee joint, which causes a painful condition including OA (Richebé et al., 2018).

OA was thought to be primarily a degenerative disease of the cartilage; however, the latest research indicates that OA has a multifactorial cause involving...
numorous factors, including trauma, mechanical forces, inflammation, biochemical reactions and metabolic derangements (Ayhan et al., 2014). The role of inflammation is not well understood and there is an ongoing debate to whether the inflammatory reaction triggers changes in OA or whether the inflammation is secondary to changes in OA (Ayhan et al., 2014).

Tumor Necrosis Factor (TNF)-α expression is associated with the progression of OA. TNF-α has been shown to be capable of regulating inflammation in an OA rat model by down regulating the Phosphoinositide 3-Kinase/protein Kinase B (PI3K/AKT) signaling pathway in synovial fibroblasts (Li et al., 2018).

Macrophage Migration Inhibitory Factor (MIF) is a pro-inflammatory cytokine produced by macrophages that may contribute to arthritis pathophysiology by promoting inflammation and angiogenesis (Llamas-Covarrubias et al., 2013).

Current approaches to OA treatment have integrated the use of biologicals that mediate the inflammatory process (Kennedy et al., 2018). Platelet-Rich Plasma (PRP) is increasingly to influence tissue regulation because it contains growth factors and high platelet levels that reduce OA-related pain (Sampson et al., 2010; Halpern et al., 2013). PRP is obtained by whole blood centrifugation, yielding a highly concentrated product containing platelets. The α-granules within the concentrated platelet solution contain growth factors and proteins that are vital to the coagulation cascade (Nurden, 2011). PRP is thought to influence the degeneration of cartilage by altering autophagy in chondrocytes. Aging cartilage gradually loses its reversible quiescence and its ability to self-renew (Chakkalakal et al., 2012).

In this study we used clinical and radiological scores to determine the potential therapeutic effects of PRP in patients with varying severities of knee OA. In addition, biochemical assays of TNF-α and MIF levels in synovial fluid were performed and their correlations with both pain and radiological scores were assessed among these patients.

Patients and Methods

Study Design and Participants

This prospective study was conducted on 90 patients with knee OA, recruited from Orthopedic Outpatient Clinics, at Qena University Hospital-South Valley University- Egypt, from February 1st, 2019 to January 31st, 2020. Based on radiological assessments, the patients were categorized into three groups (mild, moderate and severe OA) according to OA severity using MRI Osteoarthritis Knee Score (MOAKS). Each group consisted of 30 patients.

The exclusion criteria were as follows: Polyarticular disease; knee arthroscopy in the previous year; HA or steroid IA penetration in the preceding 3 months; history of infectious disease and autoimmune disorders such as diabetes, rheumatoid arthritis, hematologic diseases (coagulopathy), serious cardiovascular diseases, infections or immunodepression; anticoagulant therapy or an anti-aggregating agent; use of nonsteroidal anti-inflammatory drugs 2 weeks prior to blood collection; and < 10 g/dL of hemoglobin (Taniguchi et al., 2018).

Clinical Assessments

A detailed medical history and thorough clinical examination from every subject included age, gender, weight, height, calculation of Body Mass Index (BMI) (kg/m²). The BMI was classified as underweight (BMI <18.5 kg/m²), normal weight (18.5-24.9 kg/m²), overweight (25-29.9 kg/m²), obesity class 1 (30-34.9 kg/m²), obesity class 2 (35-39.9 kg/m²), extreme obesity class 3 (>40 kg/m²) (Dwyer et al., 2000). Local examinations of both knee joints and assessments of knee joint pain for each patient were conducting using the Visual Analog Scale (VAS) score (Katz and Melzack, 1999). OA diagnosis was based on visual identification of eburnation on the articular surfaces of the distal femur, proximal tibia, or patella, right or left. Eburnation is a sclerotic, ivory-like subchondral bone reaction that occurs from bone-on-bone contact at sites exposed to advanced cartilage erosion (Rogers and Dieppe, 2003; Pritzker et al., 2006). The VAS is a common tool that uses a 10 cm scale to measure pain intensity, where 0= no pain and 10 = unable to move. Pain score assessments were performed before the beginning PRP therapy and 2 weeks after the second PRP IA injection.

Radiological Assessments of Severity of Knee OA

MRI Osteoarthritis Knee Score was used to assess the severity of OA (Hunter et al., 2011). Briefly, the following assessments were used for both bone marrow abnormality and the patella-femoral cartilage volume: Grade I (mild if the lesion involved <33% of subregional volume); grade II (moderate if the lesion involved 33-66% of subregional volume) and grade III (severe if the lesion involved > 66% of subregional volume). Synovitis was categorized as follows: Grade 1 (mild or small-fluid continuous in the retropatellar space); grade 2 (moderate or medium - slight convexity of the suprapatellar bursa) and grade 3 (severe or large-evidence of capsular distension). A meniscal desintegrity score of 0-3 applied for the amount of extrusion at four locations: Medial meniscus (medial and anterior extrusion) and lateral meniscus (medial and anterior extrusion).

Biochemical Workup

PRP preparation: The PRP used for IA injection was prepared under completely aseptic conditions. To avoid the effect of food intake on purified PRP, patients were instructed to fast for 4 h on the day of injection before blood collection. Approximately 10 mL of venous blood was drawn from the antecubital vein using an aseptic technique in an effort to avoid irritation and trauma to the
platelets. Anti-coagulated blood was obtained in five extraction tubes (2 mL each), containing sodium citrate. The tubes were then centrifuged at 2100 rpm at room temperature for 8 min to separate the citrated plasma from the buffy coat and the residual Red Blood Cells (RBCs) (Taniguchi et al., 2018). Using a pipette, the PRP situated just above the selectively precipitated RBCs but not including the buffy coat was aspirated carefully from each tube. In each affected joint, 5-mL PRP samples (from the five blood collection tubes) were used for IA injection (Dwyer et al., 2000). For each intra-articular injection new fresh PRP samples were made from the patients.

TNF-α and MIF were measured in synovial fluid samples using commercially available ELISA kits obtained from Chongqing Biospes, China (Catalog No.: BYEK3327-48T and BYEK3015) and a microplate ELISA Reader (EMR 500, USA) according to the manufacturers' instructions. To extract cell debris, synovial fluid samples were immediately centrifuged at 3000 rpm for 15 min and the supernatant was aliquoted into 1-mL cryotubes and frozen at -80°C until use. The assays were performed for the synovial fluid samples at three points: The first sample (S1) was pre-injection; the second Sample (S2) was 2 weeks after the first injection and the third Sample (S3) was 2 weeks after the second injection.

Procedure and Timing of IA PRP Injections

The patients were placed in a supine position with a 20-degree flexion of the knee. Under aseptic conditions, a 21-gauge needle was used to inject 5mL of PRP into the suprapatellar knee joint pouch, using the superolateral method with ultrasound guidance. Local anesthetics were not used. After the injection, patients were instructed to refrain from physical exercise for at least 24 h but no restriction was specified regarding activities of daily living. Three IAPRP injections were administered at 2-week intervals (Fig.1). The same physician who selected and tested the participants administered the injections (Taniguchi et al., 2018).

Statistical Analyses

IBM SPSS Statistics v.22 software was used for data analysis. The Mean ± Standard Deviation (SD), median and inter-quartile range were used for expression of quantitative data, were as the number and percentage were used for qualitative data. The Kolmogrov-Smirnov and Shapiro-Wilk normality tests were used. Independent sample T-tests were used to compare normally distributed quantitative variables between two groups, while Mann-Whitney Test used for abnormally distributed variables. One way-ANOVA was for comparison between more than two quantitative data. Post Hoc test Least Significance Difference (LSD) was used for multiple comparisons between different quantitative variables. For qualitative variables, chi-squared ($\chi^2$) and Fisher's exact tests were used. Pearson's correlation coefficient (r) test was used to correlate data. P values <0.05 were considered statistically significant.

![Fig. 1: Scheme for the study design. S1, S2 and S3 synovial fluid samples were analyzed biochemically for proinflammatory cytokines (Tumor Necrosis Factor-α and Macrophage Migration Inhibitory factor)](image-url)
Results

Demographic and Clinical Characteristics of Study Participants

The current study included 90 patients with knee OA [9 males (10%) and 81 (90%) females], with female-to-male ratio of 9:1. The subjects’ mean ± SD age was 45.5±10.6 years with an age range of 25-65 years. The mean ± SD BMI was 31.9±2.9 kg/m² with a range of 23-37kg/m². The mean duration of knee OA was 28.8±17.5 months with a range of 3-60 months, (Table1). With respect to the BMI, 7 patients (7.7%) were categorized as overweight, 69 patients (76.6%) as obesity class 1 and 14 patients (15.5%) as obesity class 2.

All patients had unilateral knee OA. The patients were categorized into three groups based on the severity of OA: 30 patients (6 males and 24 females) had mild knee OA (33.3%), 30 patients (30 females) had moderate severity (33.3%) and 30 patients (3 males and 27 females) had severe OA (33.3%). There were no significant differences with respect to the mean age (46.8±10.2, 46±12.3 and 43.6±10.1 years, respectively), BMI (31±2.4, 32.1±2.1 and 32.6±3.9, respectively), or sex p>0.05 for all, (Table1).

Serial Synovial Fluid Levels of TNF-α as Regards Time of IA PRP Injection and Severity of Knee OA

The mean synovial TNF-α values were significantly higher (S1, pre-injection) in patients with severe OA (144.8±13.6 pg/mL) than in those with either mild (35.5±7.4 pg/mL) or moderate OA (97.3±31.7 pg/mL), p<0.05 for all (Fig. 2A). Significantly lower synovial TNF-α levels were observed 2 weeks after the second IA injection of PRP (S3) in patients with mild, moderate or severe knee OA (9.3±5.4, 44.8±11.5 and 59.7±3.9, respectively) when compared with both the pre-injection synovial fluid levels (35.5±7.4, 97.3±31.7 and 144.8±13.6, respectively) and levels 2 weeks after the first injection (S2) (17.8±7.5, 64.6±15.3 and 82.4±4.3, respectively), p<0.05 for all (Table2).
Fig. 2: Comparison of the mean synovial fluid levels of Tumor Necrosis Factor (TNF)-α (A) and Macrophage Migration Inhibitory Factor (MIF) (B) relative to the severity of knee Osteoarthritis (OA). ANOVA Post Hoc test (Fisher’s LSD) was used.

Table 1: Demographic data of the included patients with knee osteoarthritis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total patients (n = 90, 100%)</th>
<th>Mild OA (n = 30, 33.3%)</th>
<th>Moderate OA (n = 30, 33.3%)</th>
<th>Severe OA (n = 30, 33.3%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>9 10%</td>
<td>6 20%</td>
<td>0 0%</td>
<td>3 10%</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>81 90%</td>
<td>24 80%</td>
<td>30 100%</td>
<td>27 90%</td>
</tr>
<tr>
<td>Age(years)</td>
<td>Mean ± SD 45±10.6</td>
<td>46.8±10.2</td>
<td>46±12.3</td>
<td>43.6±10.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration (months) Mean ± SD 28.8±17.5</td>
<td>33.3±18.1</td>
<td>26.3±15.6</td>
<td>26.9±19.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BM(kg/m²) Mean ± SD 31.9±2.9</td>
<td>31±2.4</td>
<td>32.1±2.1</td>
<td>32.6±3.9</td>
<td></td>
</tr>
</tbody>
</table>

p1 = mild Vs. moderate knee OA groups; p2 = mild vs. severe knee OA groups; p3 = moderate vs. severe OA groups. ANOVA and post-Hoc test (LSD: Least Significant Difference) was used as the data were normally distributed.
Table 2: Comparison of synovial fluid levels of TNF-α at different durations after the IA PRP injection in patients with knee OA

<table>
<thead>
<tr>
<th></th>
<th>S1 (n = 30)</th>
<th>S2 (n = 30)</th>
<th>S3 (n = 30)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild OA</td>
<td>35.5±7.4</td>
<td>17.8±7.5</td>
<td>9.3±5.4</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>(Min.-Max.)</td>
<td>(27-44)</td>
<td>(6.25-30)</td>
<td>(4.5-13.56)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate OA</td>
<td>97.3±31.7</td>
<td>64.6±15.3</td>
<td>44.8±11.5</td>
<td>0.002</td>
<td>&lt; 0.001</td>
<td>0.048</td>
</tr>
<tr>
<td>(Min.-Max.)</td>
<td>(52-134)</td>
<td>(44-91)</td>
<td>(32-64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe OA</td>
<td>144.8±13.6</td>
<td>82.4±4.3</td>
<td>59.7±3.9</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(Min.-Max.)</td>
<td>(127-166.25)</td>
<td>(76-89)</td>
<td>(53.13-66)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P1 = S1 vs. S2; P2 = S1 vs. S3; P3 = S2 vs. S3. ANOVA and post-Hoc test (LSD: Least Significant Difference) was used as the data were normally distributed.

N.B: TNF-α: Tumor Necrosis Factor-α; PRP: Platelet Rich Plasma; OA: Osteoarthritis; SD: Standard Deviation; S1: First synovial fluid sample assay (before IA injection of PRP); S2: Second synovial fluid sample assay (2 weeks after the first IA injection of PRP); S3: Third synovial fluid sample assay (2 weeks after the second IA injection of PRP); Min.: Minimum value; Max.: Maximum value

Table 3: Comparison of synovial fluid levels of MIF at different durations after the IA PRP injection in patients with knee OA

<table>
<thead>
<tr>
<th></th>
<th>S1 (n = 30)</th>
<th>S2 (n = 30)</th>
<th>S3 (n = 30)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild OA</td>
<td>7.7±1.5</td>
<td>4.9±0.9</td>
<td>1.25±0.9</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(Min.-Max.)</td>
<td>(5.5-10)</td>
<td>(4-6)</td>
<td>(0.25-3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate OA</td>
<td>47.8±14.9</td>
<td>25.3±7.9</td>
<td>10.6±2.6</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>(Min.-Max.)</td>
<td>(20.3-68)</td>
<td>(10.5-37.5)</td>
<td>(6.25-14.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe OA</td>
<td>315.1±23.2</td>
<td>107.7±12.3</td>
<td>82.9±8.2</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>(Min.-Max.)</td>
<td>(281-364)</td>
<td>(90-127)</td>
<td>(72.57-96.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P1 = S1 vs. S2; P2 = S1 vs. S3; P3 = S2 vs. S3. ANOVA and post-Hoc test (LSD: Least Significant Difference) was used as the data were normally distributed.

N.B: MIF: Macrophage Migration Inhibitory factor; PRP: Platelet Rich Plasma; OA: Osteoarthritis; SD: Standard Deviation; S1: First synovial fluid sample assay (before IA injection of PRP); S2: Second synovial fluid sample assay (2 weeks after the first IA injection of PRP); S3: Third synovial fluid sample assay (2 weeks after the second IA injection of PRP); Min.: Minimum value; Max.: Maximum value

The mean levels of synovial MIF were significantly higher (S1, pre-injection) among patients with severe OA (315.1±23.2 μg/L) than among those with mild (7.7±1.5 μg/L) or moderate OA (47.8±14.9 μg/L), p<0.05 for all (Fig. 2B). Significantly lower synovial TNF-α levels were observed 2 weeks after the second IA injection of PRP (S3) in patients with mild, moderate, or severe knee OA (1.25±0.9, 10.6±2.6 and 82.9±8.2 μg/L, respectively) compared with pre-injection synovial fluid levels (7.7±1.5, 47.8±14.9 and 315.1±23.2, respectively) and levels 2 weeks after the first injection (S2) (4.9±0.9, 25.3±7.9, and 107.7±12.3 μg/L, respectively); p<0.05 for all (Table 3).

Using Pearson's correlation coefficient among patients with knee OA (n = 90), there was significant positive correlation between the synovial fluid TNF-α and MIF levels in S1 (r = 0.815, p = 0.000), S2 (r = 0.783, p = 0.000) and S3 (r = 0.750, p = 0.000).

Effects of IA Injection of PRP on Pain Score Among Patients with Knee OA

Significantly lower median pain score values when assessed 2 weeks from the second IA injection in patients with mild, moderate or severe knee OA (2, 2 and 5, respectively) compared with the pre-injection pain scores (3, 6 and 9, respectively), p<0.05 for all (Table4).

The mean levels of synovial MIF were significantly higher (S1, pre-injection) among patients with severe OA (315.1±23.2 μg/L) than among those with mild (7.7±1.5 μg/L) or moderate OA (47.8±14.9 μg/L), p<0.05 for all (Fig. 2B). Significantly lower synovial TNF-α levels were observed 2 weeks after the second IA injection of PRP (S3) in patients with mild, moderate, or severe knee OA (1.25±0.9, 10.6±2.6 and 82.9±8.2 μg/L, respectively) compared with pre-injection synovial fluid levels (7.7±1.5, 47.8±14.9 and 315.1±23.2, respectively) and levels 2 weeks after the first injection (S2) (4.9±0.9, 25.3±7.9, and 107.7±12.3 μg/L, respectively); p<0.05 for all (Table 3).

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**Therapeutic Effects of IA Injection of PRP on MOAKS in Patients with Knee OA**

Comparing MOAKS pre-injection value versus that two weeks from the second IA injection of PRP, there was significant improvement in bone marrow lesions among patients with only mild knee OA (p<0.05) and a significant improvement in synovitis in both patients with mild or moderate knee OA (p<0.05 for both), (Fig. 3A,3B and Table5).

There were no significant improvements in either patello-femoral cartilage volume or meniscal disintegration in various severities of knee OA, p>0.05 for all (Fig. 3C and Table 5).

**Correlations of Pain Scores with Serial Synovial TNF-α and MIF Levels**

Using Pearson's correlation coefficient, the included patients with knee OA (n = 90) showed significantly positive correlations between pre-injection synovial fluid TNF-α levels (S1) and the pre-injection pain score (r = 0.849, p = 0.000), (Fig. 4A) and between synovial fluid TNF-α levels 2 weeks after the second injection (S3) and the post-injection pain score (r = 0.536, p = 0.000), (Fig. 4B).
Fig. 3: MRI analysis of knee joint before the first IA injection of PRP and 2 weeks after the second IA PRP injection. MRI signals showed significant improvements in synovitis (A) and bone marrow lesions (B) with no improvement in cartilage loss (C) compared with the pre-injection knee joint.
Fig. 4: Correlation of the pain score with synovial TNF-α levels in patients with knee osteoarthritis (n=90) both pre-injection (A) and 2 weeks after the second injection (B). Pearson correlation was used.

Table 4: Effects of the IA injection of PRP on pain score in patients with knee OA of varying severities

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Pain score</th>
<th>Pre-injection</th>
<th>2 weeks after the second injection</th>
<th>MW*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild OA (n = 30)</td>
<td>Median</td>
<td>3</td>
<td>2</td>
<td>9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>2-4</td>
<td>1-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate OA (n = 30)</td>
<td>Median</td>
<td>6</td>
<td>2</td>
<td>11</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>5-7</td>
<td>1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe OA (n = 30)</td>
<td>Median</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>8-9</td>
<td>3-7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IA: Intra-Articular; PRP: Platelet-Rich Plasma; OA: Osteoarthritis; IQR: Inter-Quartile Range; *MW: Mann-Whitney Test was used.
Fig. 5: Correlations of the pain score with synovial MIF levels in patients with knee osteoarthritis (n = 90) both pre-injection (A) and 2 weeks after the second injection (B). Pearson correlation was used.

Table 5: Comparison of the therapeutic effects of the IA injection of PRP on MOAKS in patients with knee OA

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Pre-injection</th>
<th>2 weeks after the second IA injection</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patello-femoral cartilage volume (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild OA (n = 30)</td>
<td>0.24±0.04</td>
<td>0.22±0.05</td>
<td>0.227</td>
</tr>
<tr>
<td>Moderate OA (n = 30)</td>
<td>0.55±0.10</td>
<td>0.55±0.10</td>
<td>1.0</td>
</tr>
<tr>
<td>Severe OA (n = 30)</td>
<td>0.71±0.08</td>
<td>0.70±0.08</td>
<td>0.677</td>
</tr>
<tr>
<td>Meniscal disintegrity (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild OA (n = 30)</td>
<td>1.0±0.0</td>
<td>1.0±0.0</td>
<td>----</td>
</tr>
<tr>
<td>Moderate OA (n = 30)</td>
<td>2.0±0.0</td>
<td>2.0±0.0</td>
<td>----</td>
</tr>
<tr>
<td>Severe OA (n = 30)</td>
<td>3.0±0.0</td>
<td>2.1±1.4</td>
<td>0.280</td>
</tr>
<tr>
<td>Bone marrow abnormality (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild OA</td>
<td>0.30±0.02</td>
<td>0.19±0.07</td>
<td>0.001</td>
</tr>
<tr>
<td>Moderate OA</td>
<td>0.45±0.13</td>
<td>0.41±0.18</td>
<td>0.684</td>
</tr>
<tr>
<td>Severe OA</td>
<td>0.75±0.06</td>
<td>0.73±0.06</td>
<td>0.579</td>
</tr>
<tr>
<td>Synovitis (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild OA</td>
<td>0.80±0.79</td>
<td>0.02±0.01</td>
<td>0.0393</td>
</tr>
<tr>
<td>Moderate OA</td>
<td>1.80±0.91</td>
<td>0.03±0.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Severe OA</td>
<td>1.81±1.54</td>
<td>0.03±0.0</td>
<td>0.143</td>
</tr>
</tbody>
</table>

N.B: IA (Intra-Articular); OA (Osteoarthritis); PRP (Platelet-Rich Plasma); MOAKS: MRI Osteoarthritis Knee Score; SD: Standard deviation. Independent sample T-test was used.
Also, there were significantly positive correlations between pre-injection synovial fluid MIF levels (S1) and the pre-injection pain score ($r = 0.881, p = 0.000$), (Fig. 5A) and between synovial fluid TNF-α levels 2 weeks after the second injection (S3) and the post-injection pain score ($r = 0.677, p = 0.000$), (Fig. 5B).

**Therapeutic Outcomes of IA PRP Injection in Patients with Varying Severities of Knee OA**

Comparing the therapeutic efficacy of the IA PRP injection in patients with knee OA, there was improvement (indicated by lower VAS score) in all patients with mild knee OA (30 cases, 100%), where as there were improvements in pain scores in 15 patients (50%) with knee OA showed improvement and in only six patients (20%) with severe knee OA (Table 6).

**Discussion**

Knee Osteoarthritis (OA) in subjects over 50 years is a major cause of pain and impairment and significantly affects physical activity and quality of life (Conrozier et al., 2019). The present study evaluated the therapeutic effects of IA PRP injection in patients with knee OA using pain score, MOAKS and biochemical assays of the synovial fluid cytokines TNF-α and MIF.

Obesity or being overweight has long been recognized as important risk factors for OA, particularly knee OA (Powell et al., 2005). This study included predominantly female subjects with female- to- male ratio 9:1. Moreover, there was higher frequency of patients categorized as obesity class 1 followed by obesity class 2. Those categorized as overweight represented the least number of patients with knee OA. This corresponded with the findings of previous studies (Felson, 1990; Spector et al., 1994; Snijders et al., 2011; Hawamdeh and Al-Ajouni, 2013; Zheng and Chen, 2015; Wallace et al., 2017). The reason for the predominance of females may be multifactorial and involves structural variations, prior trauma, genetic and hormonal disorders. Men had significantly higher total tibial and patella cartilage volumes than women. Women also had a significantly higher prevalence of baseline patellar cartilage defects. Over time females exhibited more volume loss in the knee cartilage than males (Hame and Alexander, 2013). One mechanism by which obesity leads to knee OA is the possibly increased mechanical load on the joint. Knee overload might cause synovial joint breakdown and failure of ligaments and other structural support (Hame and Alexander, 2013).

Although PRP has been approved as an agent for knee OA therapy, studies of PRP in knee OA have found consistently more positive results than hyaluronic acid, other Intra-Articular (IA) injections and placebo compared with other musculoskeletal tissues (Dai et al., 2017; Huang et al., 2018 and O'Connell et al., 2019). Although the causes of knee OA are not understood completely, laboratory and clinical evidence suggests that inflammatory cytokines contribute to its pathogenesis (Chiu et al., 2011; Kapoor et al., 2011). The present study revealed significantly higher synovial fluid inflammatory cytokine (TNF-α and MIF) levels (S1, pre-injection) among patients with severe OA than in those with mild and moderate OA. Significantly lower synovial TNF-α and MIF levels were observed 2 weeks after the second IA injection of PRP (S3) in patients with mild, moderate or severe knee OA than from both the pre-injection synovial fluid and 2 weeks after the first injection (S2). These results indicate that PRP treatment for patients with knee OA yielded beneficial effects in regulating inflammatory factors. Inflammatory cytokines form a complex regulatory signaling network in femoral head osteonecrosis, which is mediated by different intracellular kinase signaling pathways to regulate the recruitment, stimulation and activation of autoimmune cells (Lebouvier et al., 2015). In accordance, Zhang et al. (2016) reported that MIF levels in synovial fluid, but not in serum, were associated independently with the severity of self-reported pain in patients with OA. Huang et al. (2018) reported significantly down regulated plasma concentrations of cytokines involving TNF-α after PRP treatment in patients with knee OA.

Our results revealed significantly positive correlations between synovial fluid TNF-α and MIF levels in patients with knee OA. MIF is a potent pro-inflammatory cytokine that causes the release of many inflammatory cytokines, Including interferon (IFN)-γ, Interleukin (IL)-1β, 6, 8 and TNF-α by initiating an inflammatory cascade (Leech et al., 1999).

We evaluated the therapeutic effects of IA PRP injections on the pain score and MOAKS in patients with knee OA. There were significantly lower pain score values when assessed 2 weeks after the second IA injection in patients with knee OA and significant improvements in synovitis and bone marrow lesions primarily for mild knee OA and to a lesser extent for moderate OA. Unfortunately, the treated patients did not show any significant improvements in the patella-femoral cartilage volume or meniscal disintegration. Similarly,
Study Limitations

The lack of long-term follow up on the patients, which should be done in future studies and a small sample size were the main limitations of our study. Lack of control group was because of ethical concerns as it is difficult to take synovial fluid samples from healthy volunteers. Lack of characterization of the PRP samples that were injected, specifically, the platelet counts, the percentages of white cells, red blood cells and other cellular components within the PRP and the correlation of these counts to the findings of the various synovial fluid inflammatory markers that were obtained were also the other study limitations that will be approached in future related researches.

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Authors’ Contributions

Mohammed H. Hassan: Study concept and design, blood sampling, biochemical and laboratory assays, PRP preparation, statistical analysis, literature research, first manuscript drafting.

Sawsan Abuhamdah: Statistical analysis, literature research.

Tahia H. Saleem: Literature research.

Elsayed Said: Study concept and design; clinical evaluation of the cases.

Nehal Ashraf Zaki: PRP preparation, Statistical analysis.

Ghada M. Abdelrazek: Radiological evaluation of patients, literature research, statistical analysis.

Safaa Y. Salim: Literature research, biochemical and laboratory assays, PRP preparation, statistical analysis.

Hamdy Tammam: Study concept and design; clinical evaluation of the cases.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are presented within the manuscript.

Ethics Approval and Consent to Participate

Prior to start of the study, approval from the Ethics Committee of Faculty of Medicine, South Valley University, Qena, Egypt, was obtained. The ethical approval code (SVU-MED-MBC004-19-1). The study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from every patient.
Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Abbreviations


References


