ORIGINAL ARTICLE

INTRA-ARTICULAR AUTOLOGOUS PLATELET LYSATES PRODUCE POSITIVE MRI STRUCTURAL CHANGES IN EARLY AND INTER-MEDIATE KNEE OSTEOARTHROSIS

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ABSTRACT

PURPOSE: The purpose of this study is to explore if autologous platelet lysate (PL) delivered by percutaneous intra-articular method can induce positive structural changes as detected by magnetic resonance imaging (MRI) in patients with early and intermediate knee osteoarthrosis (KOA). **DESIGN:** Open label prospective study. **MATERIALS AND METHODS:** 15 adult patients of either gender with unilateral or bilateral early to intermediate KOA and imaging findings whether radiography or MRI of degenerative changes in the joint of grade I or grade II Kellgren scale with an age range from 35-70 years were included in this study. Autologous PL was obtained by physical method. The PL was given in the knee joint via percutaneous route every 3 weeks for a total of three injections. All patients under went baseline and at 12 months MRI evaluation to assess and compare cartilage morphology and thickness at baseline and at fifty two weeks. Plain radiographs were performed at baseline and at 12 months. Here we report imaging findings of these patients after completing MRI evaluation. **RESULTS:** Quantitative MRI demonstrated significant improvement in cartilage thickness for both tibial plate (p= 0.044) and femoral plate (p= 0.028) at twelve months following PL injection. **CONCLUSION:** Percutaneous intra-articular PL given to patients with early and intermediate KOA produces significant improvement in the thickness of the knee cartilage as measured by MRI.

Keywords: Osteoarthrosis, platelets lysate, knee, cartilage, MRI.

Abbreviations: KOA-Knee Osteoarthritis, PL-Platelet Lysate, MRI-Magnetic Resonance Imaging, OA-Osteoarthritis, PRP-Platelet Rich Plasma, KOOS-Knee Osteoarthritis Disability and Outcome Score, PPP-Platelet Poor Plasma, SD-Standard Deviation, SPSS-Statistical Package for the Social Sciences, Inc.-Incorporation, IKDC-International Knee Documentation Committee, HA-Hyaluronic Acid

Introduction

Knee osteoarthrosis (KOA) is a widely spread clinical problem in old population.[1,2] It has important social and economic burden affecting millions of people. It is especially common in the Middle East.[3,4] The prevalence of KOA varies significantly depending on the definition or criteria used.[5-7] In Framingham Study, the prevalence of radiographic KOA in individuals over 45 years was 19.2% and 43.7%, in those over

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80 years.[1] The Dutch Institute for Public Health data showed a prevalence of KOA of 15.6% in those aged 55 and above.[8]

Cartilage loss is considered to be the dominant element in osteoarthrosis (OA). Interventional modern approaches to induce the repair of the damaged cartilage are currently being used with good clinical success such as the use of platelet rich plasma (PRP) or platelet lysates (PL).[3,5-6]

In a previously published paper of et al., 2014,[4] we assessed the clinical effect of PL on KOA by using the Knee osteoarthritis and Disability outcome Score (KOOS) non-normalized score, whileinthis studywe looked at patients' MRI images at baseline and after 12 months to see if PL had any effect on the thickness of the knee cartilage.

Material and Methods

15 adult patients were enrolled from February 2012 to July 2012. 10 males and 5 females with mean age 50.4 years (Range 44-63 years). All patients were followed for a minimum of 1 year and recruited from Orthopaedic department of our hospital. Their MRI images at baseline and at 12 months were analysed and were included in this study. MRI images were reviewed independently by two MRI experienced radiologists followed by a consensus to resolve any difference in interpretation

A written informed consent was obtained from every patient in accordance with the declaration of Helsinki. The study was approved by review board and ethics committee at our institution, while (KOOS) non-normalized was adopted using a validate Arabic language format.

Inclusion and Exclusion criteria:

Inclusion criteria involved adult patients of either gender from 35-70 years, history of chronic pain (at least 4 months) or swelling of 1 or 2 knees, imaging findings whether via radiography or MRI of degenerative changes in the joint (grade I or II on the Kellgren scale), baseline test and retest MRI at 12 months after serial percutaneous intra-articular PL injections.

Exclusion criteria involved rheumatoid arthritis, active autoimmune disease, uncontrolled diabetes mellitus, ongoing infections, active cancer, major axial deviation deformity (varus or valgus), haemorrhagic diseases (coagulopathies), severe cardiovascular diseases, immune suppression, patients on therapy with anticoagulants or inhibitors of platelet aggregation, use of non-steroidal anti-inflammatory drugs (NSAIDs) 5 days prior to blood donation and patients with haemoglobin values < 11g /dl and platelets values < 150 000/µic L.

MRI acquisition:

MRI images were obtained using 3.0 Tesla scanner (Magnetom, Verio 3T, SIEMENS, Erlangen, Germany) with knee coil (8 channels). The following sequences were acquired (in supine position) and used for image analysis: sagittal T1 spin echo (TR/TE 500/11, 4 mm, 192 X 256, FOV 140), sagittal T2 turbo spin echo (2500/89, 4 mm,192 X 256, FOV 140), sagittal T1 FLASH 3D (58/6, 2 mm, 205 X 320, FOV 140), axial T1 FLASH 3D (58/6, 2 mm, 205 X 320, FOV 140), coronal T2 FATSAT turbo spin echo (2500/89, 4 mm, 192 X 256, FOV 140). Siemens workstation Syngo was used with software Syngo MMWP version VE36A for image analysis.

Image analysis:

The femoro-tibial cartilage was divided into four plates by anatomic location: medial femoral, lateral femoral, medial tibial and lateral tibial. Three measurement points were taken for each plate: anterior, median and posterior. The mean cartilage thickness and standard deviation (SD) were calculated for tibial and femoral plates at baseline and at 52 weeks.

Platelet Lysate Preparation:

Blood samples (20 ml) were collected in the day of intra-articular injection in each event. The autologous blood was centrifuged at 1000 rpm for 13 minutes to obtain PRP. A second centrifugation was performed for the PRP at 4000 rpm for 10 minutes to acquire platelet pellet and supernatant platelet poor plasma (PPP). The pellet was re-suspended in 5 ml PPP, the suspended pellet was frozen twice at - 80°C each time for 30 minutes. The suspension was melted between the first and second freeze, the melted suspension was centrifuged at 4000 rpm, and the supernatant was obtained and filtered with 0.2 micro-millimetre filters. The filtered productwas drawn in a sterile syringe and used for intra-articular injection. Platelet count in the first blood sample and PRP sample were obtained. The platelets concentration in the sample prepared for PL was on an average 5-

6 times more than in the initial whole blood platelet count. Platelet count in whole blood of all subjects ranged between 180 and 302 *10^9 / L compared with 1000 and 17000 *10^9/ L in the modified prepared PL. PL were prepared at the Haemostasis and thrombosis Laboratory and cell therapy centre at our university.

Treatment procedure and follow up:

After the first evaluation including baseline MRI and KOOS non-normalized score, filtered PL was given every 3 weeks on day 0, 21, and 42 and intra articular injection was given blindly using a lateral approach; all injections were administered by a single consultant orthopaedic surgeon without any complications. KOOS non-normalized score questionnaire was obtained on weeks 3, 6, 12, 32 and 52 and MRI was repeated after 1 year.

Statistical analysis:

Statistical package for social sciences (SPSS, version 16.0, spss Inc, Chicago, IL) wasapplied and data is expressed as mean and SD. Student t test is used to confirm the significance of differences between mean values of 2 continuous variables andconfirmed by nonparametric Mann-Whitney U test. One-way of analysis of variance was applied for comparison of several group means and to determine the presence of significant differences between the group means. The level P < 0.05 was considered as the cut-off value for significance.

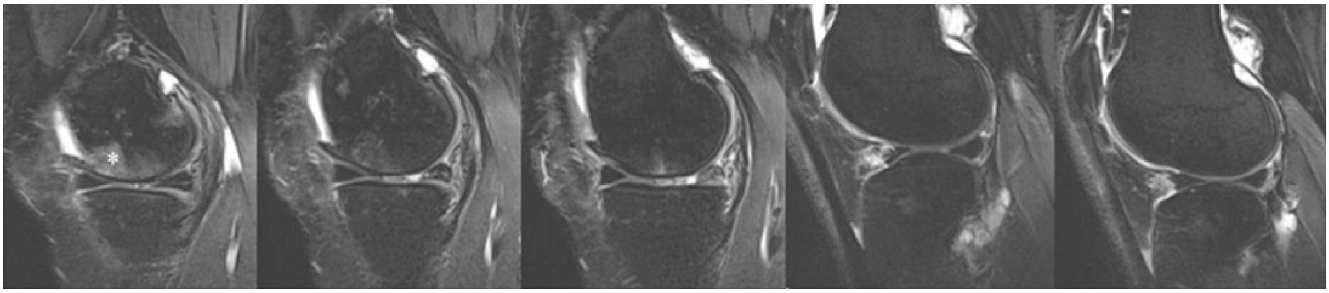
MRI results:

As shown in (Tab. 1), the mean and SD of cartilage thickness before autologous PL injection were (13.23 ± 2.52) for tibial plate and (14.23 ± 2.68) for femoral plate. After PL injection at 52 weeks the mean and SD increased to (13.6 ± 2.62) for tibial plate and (14.66 ± 2.68) for femoral plates. All patients showed an increase in MRI based cartilage thickness measurement which was significant (p<0.05) (Fig. 1A, 1B and 1C), while no radiographic changes could be seen in any of the study patients.

Tibial plate	Mean T2 Baseline/ mm	13.02	Mean T2/ at 12 months mm	13.6	P*0.0437
	SD	2.517	SD	2.62	
	SE	0.649	SE	o.676	
Femoral plate	Mean T2 Base line	14.226	Mean T2/ at 12 months/ mm	14.66	P* 0.028
	SD	2.684	SD	2.681	
	SE	0.693	SE	0.69	
KOOS	@∓/SD	76.35/ 12.2	ß∓/SD	48.7/ 11.1	P*<0.0001

α: Plate thickness is the mean thickness of 3 point measurements: anterior, medial and posterior parts of the plate.
* P as calculated by 2 tailed.
T1 measurement of plate thickness in mm at baseline
T2 measurements of plate thickness in mm at the end of treatment
@ KOOS score non-normalized at baseline
ß KOOS score non-normalized at 12 months.

Table 1: Mean plate thickness$^{\alpha}$ in mm as measured by MRI in 15 patients at baseline and post treatment at 12 months



(Figure 1A,B,C): Sagittal fat suppressed T2 images for a female patient with knee osteoarthrosis
Figure 1A: Baseline images of a 56 years old female with a non-normalized KOOS result of 76 showing full thickness cartilage defect at the central weight bearing part of medial femur and tibia. In addition there was an adjacent subchondral bone marrow hyperintense area indicating oedema (asterisk).

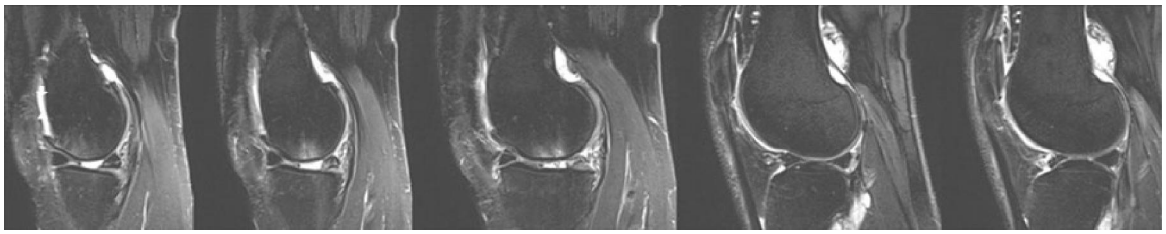


Figure 1B: One year following treatment images for the same patient with a decrease in non-normalized KOOS result of 53 showing a sizable increase in the cartilage thickness indicating cartilage regeneration and a decrease of subchondral oedema.

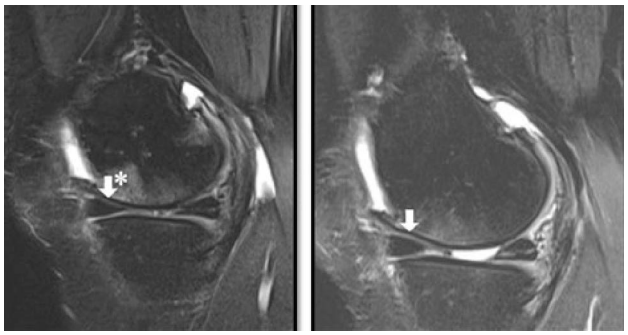


Figure 1C: Marked improvement of cartilage thickness (arrow) and bone marrow oedema (asterisk).

Discussion

Several studies have shown the usefulness of PRP in improving the clinical outcome of early stage KOA.[9,10] In a recent study Filardo G et al showed that PRP was not superior to Hyaluronic acid (HA) in the treatment of stage 0-3 KOA as measured by clinical outcome using International Knee Documentation Committee (IKDC).[11] None of these studies did any post treatment MRI evaluation, except Halpern B et al who tried to correlate the clinical outcome with MRI; but patients clinical improvement did not match their MRI results.[12]

Since we were the first to report the effects of PL in early KOA,[4] we present here the positive effect of PL on the thickness of the knee cartilage. We followed 15 patients, who completed MRI at baseline and at 12 months, and we compared the thickness in both images and found an improvement, also we compared the clinical outcome as measured by KOOS- non-normalized score with that of MRI in these patients. The clinical improvement outcome was highly significant, while the MRI improvements (Fig. 1C) were modest but statistically significant.

The mechanism by which PRP and by inference PL improve KOA is still not clearly understood. In an ovine model, PRP produced proliferation of autologous chondrocytes and mesenchymal stem cells.[13] PRP releasate when added to chondrocytes from patients with KOA inhibit inflammatory processes.[14] These include diminish nuclear factor-B activation, inhibition of interleukin 1ß and aggrecan gene expression.

The limitation of our study and that of Halpern B et al is the small number of patients and the lack of long term follow up. Furthermore, we used the PL while Halpern B et al used PRP and comparison between the two products has not been doneyet, and it is not known whether the two are similar. We have no clear explanation for our finding and may be our product has less inflammation- inducing molecules since we depleted the white cells and the platelet membranes in the process of preparation of PL.

We believe a larger number and long term follow up study is needed to confirm our finding with additional molecular studies to explain our results.

Limitation of the study were lack of standardization of MRI cartilage thickness measurements, limited number of patients and limited financial supply

This study suggests that autologous PL may play a role in improving the knee cartilage thickness measured by MRI in early on set of KOA. The MRI improvement didn't correlate with the clinical outcome despite being statistically significant.

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