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Clinical and laboratory findings following transplantation of allogeneic adipose-derived mesenchymal stromal cells in knee osteoarthritis, a brief report

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ABSTRACT

Background: Mesenchymal stromal cells (MSCs) injection has been proposed as an innovative treatment for knee osteoarthritis (KOA). Since, allogeneic MSCs can be available as off-the-shelf products, they are preferable in regenerative medicine. Among different sources for MSCs, adipose-derived MSCs (AD-MSCs) appear to be more available.

Methods: Three patients with KOA were enrolled in this study. A total number of 100×10^6 AD-MSCs was injected intra-articularly, per affected knee. They were followed up for 6 months by the assessment of clinical outcomes, magnetic resonance imaging (MRI), and serum inflammatory biomarkers.

Results: The primary outcome of this study was safety and feasibility of allogeneic AD-MSCs injection during the 6 months follow-up. Fortunately, no serious adverse events (SAEs) were reported. Assessment of secondary outcomes of visual analogue scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and knee osteoarthritis outcome score (KOOS) indicated improvement in all patients. Comparison between baseline and endpoint findings of MRI demonstrated a slight improvement in two patients. In addition, decrease in serum cartilage oligomeric matrix protein (COMP) and hyaluronic acid (HA) indicated the possibility of reduced cartilage degeneration. Moreover, quantification of serum interleukin-10 (IL-10) and interleukin-6 (IL-6) levels indicated that the host immune system immunomodulated after infusion of AD-MSCs.

Conclusion: Intra-articular injection of AD-MSCs is safe and could be effective in cartilage regeneration in KOA. Preliminary assessment after six-month follow-up suggests the potential efficacy of this intervention which would need to be confirmed in randomized controlled trials on a larger population.

Trial registration: This study was registered in the Iranian registry of clinical trials (<https://en.irct.ir/trial/46>) in 24 April 2018 with identifier IRCT20080728001031N23.

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Allogeneic mesenchymal stromal cells; cartilage regeneration; inflammatory biomarkers; knee osteoarthritis; regenerative medicine

Introduction

Osteoarthritis (OA), a chronic and progressive disease, usually affects the articular cartilage and subchondral bones¹. OA is usually seen in joints that bear weight or are under pressure. Epidemiological studies showed that the knee joint is more susceptible to this pathology compared to other joints². It is estimated that 14 million people in the US suffer from symptomatic knee osteoarthritis (KOA) and it is expected that up to

25% of the US population could be affected by 2040³. Studies noted that women are more prone to KOA associated with advanced age, and other risk factors such as obesity and sedentary lifestyle increase the incidence and prevalence of this disease^{4,5}.

Owing to the high prevalence of KOA in the world, there are different conventional therapeutic approaches. However, proposing an approved treatment for this disease remains a challenge for medical

experts. Regenerative medicine including tissue engineering and cell-based therapies are thriving approaches that have specially been considered in bone and cartilage tissues⁶. Cartilage tissue engineering with different specific scaffolds, cells, and biologically active molecules induces the articular cartilage to repair^{7,8}. Scaffolds, especially the biological ones, have indicated efficacy in cartilage repair. However, stem cells are applied without scaffolds in most clinical trials. This technique has not only been effective in cartilage repair but also bypasses the surgical risks of scaffold implantation. There are various cell sources for cartilage regeneration applications, but most of them have drawbacks. For instance, the shortcomings of chondrocytes usage for cartilage regeneration encompass donor-site morbidity, de-differentiation of cells, and limited lifespan⁹. Mesenchymal stromal cells (MSCs) are non-hematopoietic precursor cells that can be found in 17 potential sources¹⁰. Among them, bone marrow, subcutaneous fat and umbilical cord have the top three therapeutic imminence indices in the last 10 years¹¹.

MSCs are counted as an investigational therapeutic product because of their regenerative, immunomodulatory, and paracrine properties¹². MSCs with anti-inflammatory characteristics have been proposed to prompt pain relief in KOA¹³. MSCs can differentiate into chondrocytes, which could play an essential role in cartilage repair. Thereafter, synthesis of the extracellular matrix (ECM) leads to the enhancement of neocartilage formation¹⁴. In a systematic review¹⁵ analyzing 28 clinical trials, 584 patients with KOA were treated with MSCs derived from bone marrow or adipose tissue. This review provided conclusive evidence for the safety and efficiency of autologous MSCs intra-articular injection in KOA.

Bone marrow-derived MSCs (BMSCs) and adipose-derived MSCs (AD-MSCs) have similar phenotypes. However, AD-MSCs have taken precedence over BMSCs in some studies. AD-MSCs not only could be isolated more easily, but also retain their differentiation capacities longer than BMSCs by the passage of time^{16,17}. Additionally, in a recent study¹⁸, AD-MSCs could commence a cytokine cascade that results in the modulation of host's immune system.

Autologous MSCs have become more popular, although allogeneic MSCs have been considered in the last decades due to some advantages such as more availability. Despite concerns about the host's immune response to allogeneic MSCs, these cells have brought about excellent safety in many studies^{19–21}. A few investigations have already been done for the evaluation of the safety and efficacy of intra-articular

transplantation of allogeneic AD-MSCs in KOA. Clinical outcomes and magnetic resonance imaging (MRI) in these studies represented significant pain reduction and some extent of compositional cartilage improvement^{22,23}. However, few studies have evaluated changes in laboratory findings following treatment in the context of pro-inflammatory cytokines as well as cartilage-specific biomarkers. Evaluation of these cytokines in addition to the clinical examination and imaging-based methods can provide a more accurate assessment matrix for evaluation of the progression or improvement of the disease, following this cell-based treatment²⁴.

In this paper, we have conducted an open-label pilot study to evaluate clinical and paraclinical changes following intra-articular injection of allogeneic AD-MSCs in three patients with chronic KOA.

Materials and methods

Study design and patients

The current study is phase I of an open-label clinical trial that was approved by the Ethics Committee of the Royan Institute (IR.ACECR.ROYAN.REC.1396.138) and registered in the Iranian Registry of Clinical Trials (<https://en.irct.ir/trial/46>) with ID code number: identifier IRCT20080728001031N23. The Data and Safety Monitoring Board (DSMB; Royan Institute) at the Royan Institute was in charge of monitoring the trial and independent experts advised researchers and clinicians. This research was conducted at Shafa Yahyaian hospital between April 2018 and March 2019 and was in accordance with the good clinical practice (GCP) guidelines and the latest version of the Helsinki Declaration.

Three patients aged 35–54 years were enrolled for the safety evaluation of allogeneic AD-MSCs injection in the knee joint in patients with KOA. They had mild-to-moderate KOA (Kellgren/Lawrence (K/L) grades of II/III), diagnosed by standard knee x-ray imaging with standing anterior-posterior (AP) and horizontal lateral radiographic views. The inclusion and exclusion criteria of the patients are illustrated in Table 1. Signed informed consent was obtained from all three participants after an accurate explanation of the procedure by the physicians.

An experienced orthopedic surgeon injected allogeneic AD-MSCs intra-articularly using a medial infra-patellar approach. A single dose of 100×10^6 allogeneic AD-MSCs suspended in 5 ml normal saline (NS) was applied to the affected knee of each patient with a 10 ml syringe under sterile conditions. Patients

Table 1. Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
1. 18 < Age < 65	1. BMI undefined kg/m ²
2. Kellgren-Laurence grading: II or III	2. Immune deficiency
3. VAS Index Score ≥ 3	3. Renal failure (Cr undefined mg/dL)
4. Knee joint alignment undefined degrees	4. Hepatitis B, C, HIV, HTLV1–2, CMV infections
5. Informed consent	5. Coagulopathy or using anticoagulant agents
	6. Liver malfunction (ALT or AST undefined IU/L)
	7. Uncontrolled diabetes (HbA1c > 8.5%)
	8. Heart diseases (EF < 45%)
	9. History of allergy to proteins or culture substances
	10. Pregnancy or lactation
	11. Malignancy
	12. Drug abuse
	13. Concurrent corticosteroid therapy
	14. Participating in another clinical trial
	15. Unwillingness to cooperate in the study and follow-ups

VAS, visual analogue scale; BMI, body mass index; Cr, creatinine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, hemoglobin A1c; EF, ejection fraction; CMV, cytomegalovirus; HTLV1–2, human T-lymphotropic virus; HIV, human immunodeficiency virus.

were only allowed to take acetaminophen to alleviate the potential upcoming pain.

Allogeneic AD-MSCs preparation

A healthy donor (30 years old female), whose health was confirmed after physical exam and laboratory tests, underwent liposuction surgery under local anesthesia and sterile conditions after signing informed consent. AD-MSCs were isolated and cultured in the IR-FDA GMP-certified clean room facility, CellTech Pharmed Co. All procedures followed good manufacturing practice (GMP) conditions.

The harvested abdominal subcutaneous tissue (250–300 ml) was washed with phosphate-buffered saline (PBS) which contained antibiotics and was cut into 1–2 mm sections afterward. In the first stage, a combination of collagenase enzyme 0.2% and dispase protease was added to the adipose tissue with the same volume. The mixture was incubated for 15–30 min while being shaken every 5 min, then neutralized with fetal bovine serum (FBS) 10% culture media with 1:1 ratio. After centrifugation at 2000 rpm, the cells of the stromal vascular fraction (SVF) were collected in a conical tube and passed through a 0.7 μ m mesh filter. In the next stage, the number of mononuclear cells (MNCs) in SVF was counted by a nucleocounter and 10 million MNCs were transferred into T-175 flasks containing Dulbecco's modified eagle's medium (DMEM)-F12 and FBS 10% culture media. The flasks were then kept for 3 days in the CO₂ incubator at 37 °

C. Three days later, the supernatant was removed. Then, 25 ml of the culture media was added to the flasks. Culture media of the flasks was changed every 4 days for 3 weeks. At the end of the third passage, AD-MSCs were harvested and cryopreserved in liquid nitrogen. Then, the cryopreserved AD-MSCs were thawed before each transplantation. AD-MSCs were thawed and suspended in NS solution 0.9% with a density of 100×10^6 cells/5 ml and transported in a standard cool box at a temperature of 2–4 °C to the Shafa Yahyaian hospital for intra-articular application. These allogeneic AD-MSCs were received from a GMP certified biobank in Celltech Pharmed Co. One month after AD-MSCs transplantation to the first patient and without observing any adverse events (AEs) and serious adverse events (SAEs), the second and after another one month the third patients were received injections, respectively.

The sterility of AD-MSCs was confirmed in several stages before injection by various microbial, endotoxin, and mycoplasma tests. Moreover, differentiation potential, mixed lymphocyte reaction (MLR), flow-cytometric, and immunophenotypic profiles were investigated to determine the characteristics of the AD-MSCs and verify their purity and mesenchymal nature. Fellow analysis data and microscopic images that indicate morphological characteristics of MSCs and their differentiation potential are presented in the supplementary data.

Outcome measures

AEs and SAEs were used to assess the safety profile as the main study outcome during the scheduled follow-up visits at baseline (prior to the injection), 1 week, 1, 3, and 6 months after injection. AEs and SAEs were recorded in the patients' history and physical examination through checking for swelling, warmth, redness, and any other changes felt by patients such as itching. In addition, various laboratory tests (complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hemoglobin (Hb), hematocrit (Hct), sodium (Na), potassium (K), calcium (Ca), serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), total and direct bilirubin (Bil (T,D)), alkaline phosphatase (ALP), vitamin D3 (Vit D3), fasting blood sugar (FBS^L), triglycerides (TG), cholesterol (Chol), low-density lipoprotein (LDL), high-density lipoprotein (HDL), blood urea nitrogen (BUN), creatinine (Cr), prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR), bleeding time (BT),

clotting time (CT), hepatitis B surface antibody (HBS Ab), human immunodeficiency virus (HIV), hepatitis B surface antigen (HBS Ag), hepatitis B core antibody (HBC Ab), hepatitis C antibody (HCV Ab), thyroid stimulating hormone (TSH), thyroxine (T4), urinalysis (U/A)) were carried out at baseline and 6 months after injection.

The secondary outcomes demonstrating the efficacy of this study comprised visual analogue scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and knee osteoarthritis outcome score (KOOS) questionnaires at the baseline and in 3 and 6 months after injection. Short form 36 (SF-36) health survey was another questionnaire that facilitated comparison between the health status of the patients before and 6 months after the treatment.

Additionally, the enzyme-linked immunosorbent assay (ELISA) was employed to measure the levels of various specific biomarkers (cartilage oligomeric matrix protein (COMP), hyaluronic acid (HA), C-telopeptide fragments of type II collagen (CTX-II), matrix metalloproteinase-3 (MMP-3), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α)) in patients' serum, which disclosed the impacts of AD-MSCs on alleviation of inflammation and cartilage degradation at baseline, 1 week, 1 month, 3 months, and 6 months after injection.

MRI evaluations were performed via 3 Tesla system (Magnetom Tim Trio; Siemens, Erlangen, Germany) with Standard knee MRI imaging protocol (Ax PD FS, SAG PD FS, COR PD FS, T2MAP, StarMap, Sag FSPGR BRAVO, Sag DTI 1000, Sag DTI 700). Axial, coronal, and sagittal planes were reviewed and the cartilage thickness, cartilage volume, subchondral bone edema, and joint effusion were analyzed using ITK-SNAP software.

Results

Demographic

The characteristics of the patients are illustrated in Table 2. Three female patients were identified as P1,

Table 2. Demographic characteristics of the patients.

Patients	P1	P2	P3
Age (year)	55	48	38
Gender	F	F	F
Kellgren-Lawrence grade	3	3	2
R/L	R	L	R
BMI (Kg/m ²)	30.85	30.86	26.76
Disease duration (months)	24	18	8
Potential origin of KOA	Life style	Life style	Life style

F, female; R, right knee; L, left knee; BMI, body mass index.

P2, and P3. Patients referred to the knee clinic of Shafa Yahyaian hospital 2–3 months before injection and mild-to-moderate KOA was diagnosed in all three patients by the use of plain radiographs of the knee. Based on the negative family history in all patients, any genetic inputs in the etiology of the KOA were ruled out. Furthermore, excluding the role of any trauma, post-injury development, work/career-associated etiologies, and according to the relevant questionnaires, lifestyle was recognized as the etiology of KOA in all three patients. The information on radiographic views to determine the K/L grade can be found in the supplementary data.

All three patients were followed up for 6 months after injection. No SAEs were seen in any of the patients. However, in P3, swelling and mild local pain in the injected knee were recorded 1 week after injection, which was self-limited and relieved after 4 days. Various laboratory tests were run to investigate possible inflammation, but the results did not reveal any abnormal parameters (Table 3).

Clinical outcomes

The clinical results of this study were analyzed using VAS, WOMAC, KOOS, and SF-36 questionnaires. Figure 1(a) contains total WOMAC scores for all patients. This linear diagram illustrates reduction in WOMAC scores for all patients at the end of 6 months. The decrease in WOMAC score, which is regarded to be an indicator of improvement of patients' conditions, initially occurred with a steep slope and decreased from 54, 43, and 53 to 16, 19, and 8 for P1, P2, and P3, respectively, after 3 months, and the decrease in WOMAC scores continued until 6 months. Moreover, Figure 1(b) illustrates a remarkable reduction in the VAS after 3 and 6 months of AD-MSCs injections compared to baseline for all three patients. Assessment of various subgroups of KOOS (symptoms, pain, sport and recreation, daily function, and quality of life) revealed enhancements in all three patients from the injection time-point to the last follow-up (Figure 2). The SF-36 outcomes, including eight subgroups for investigation of health status, have been illustrated in Figure 3. Similar to KOOS, a high score on SF-36 means better health state. Significant improvement in the health conditions of all patients was accomplished in all subgroups except in social functioning, which remained the same in P1 and P2 6 months after the treatment.

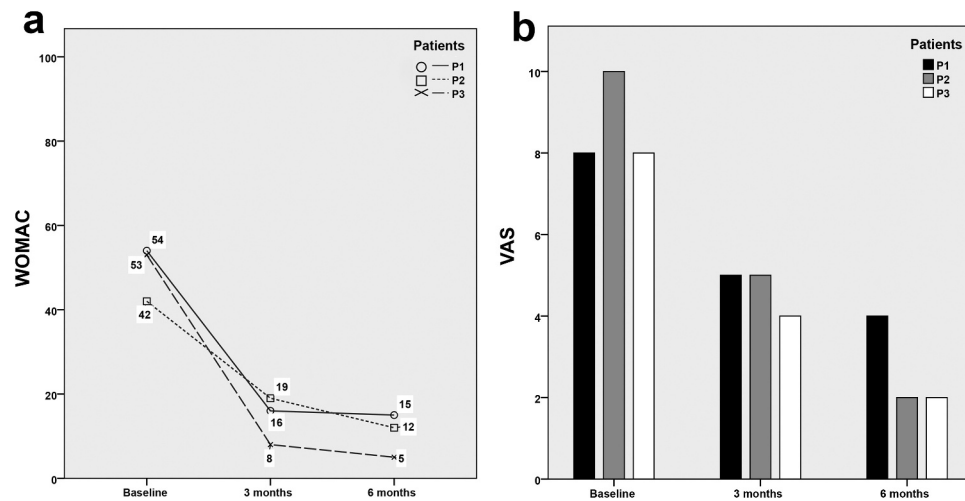


Figure 1. Changes in the (a) WOMAC and (b) VAS during the 6-months period after AD-MSCs injection. WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; VAS, visual analog scale; AD-MSCs, adipose derived mesenchymal stromal cells.

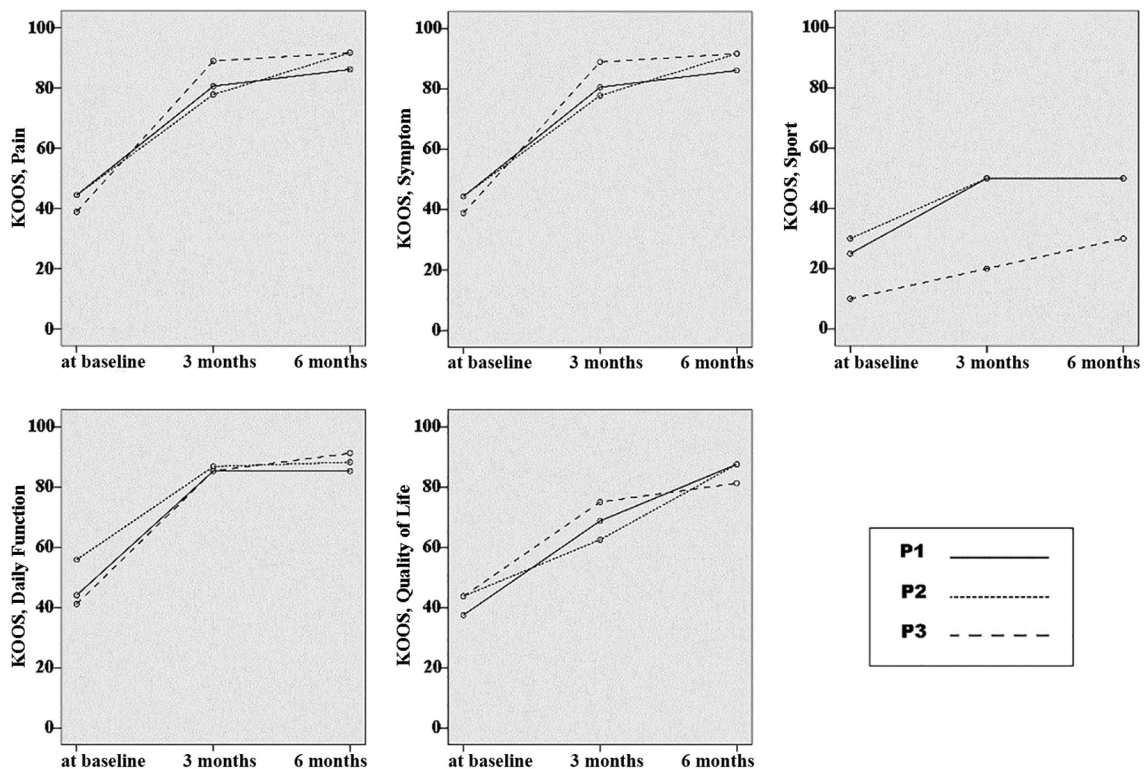


Figure 2. Changes in the five separately scored subscales of KOOS (pain, symptom, sport, daily function, and quality of life) during the 6-months follow up after AD-MSCs injection. KOOS, Knee injury and Osteoarthritis Outcome Score; AD-MSCs, adipose derived mesenchymal stromal cells.

Radiological findings

Identification of changes in cartilage volume and thickness, joint effusion, and subchondral edema was achieved by comparing the MRI findings before and 6 months after AD-MSCs therapy. Table 4 provides the changes in detail. Figure 4 illustrates the coronal views

for P2 before and after the AD. Injection of AD-MSCs not only led to an increase in the cartilage volume but also resulted in a reduction in subchondral edema and joint effusion. The MRI images of before and after treatment for P1 and P3 are presented in the supplementary data.

SF-36

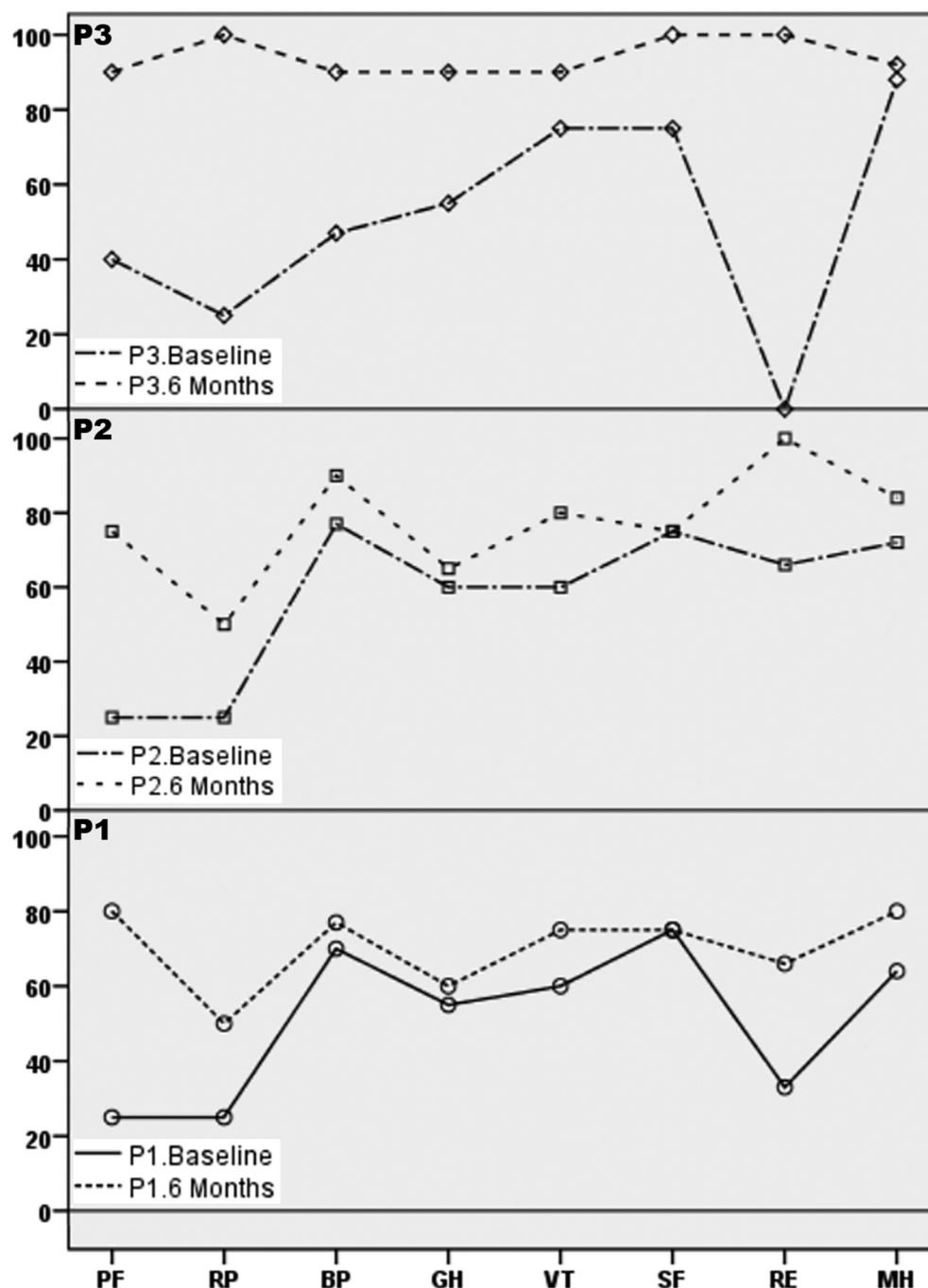


Figure 3. SF-36 outcomes in P1, P2, and P3 the baseline and 6 months after AD-MSCs injections. PF, physical functioning; RP, role limitation due to the physical health; BP, body pain; GH, general health; VT, vitality; SF, social functioning; RE, role limitation due to the emotional health; MH, mental health; SF-36, 36-Item Short Form Survey; AD-MSCs, adipose derived mesenchymal stromal cells.

Biomarker assessment

Intra-articular injection of allogeneic AD-MSCs had some systemic consequences. IL-6 levels have been analyzed (Figure 5(a)) and the results signified a reduction in its

expression 1 week after AD-MSCs injection in all patients. After a slight growth observed after four and 12 weeks, the levels of this marker decreased at the end of 6 months.

Table 3. Adverse and serious adverse events.

Patients	P1	P2	P3
AEs	-	-	Swelling of injection-site joint
Outcome	-	-	Relief
SAEs	-	-	-

AEs, adverse events; SAEs, serious adverse events.

IL-10 is another marker that was assessed in this study (Figure 5(b)). The expression of IL-10 had a notable rise after a week. While, it went through a downward trend in the following weeks. This downward trend was the same for all three patients. Figure 5(c) demonstrates the variations in COMP levels during the 6 months. The amount of COMP decreased dramatically in the first week after injection in all patients, but it approximately became constant in 12 and 24 weeks post-treatment. HA expression levels have been shown in Figure 5(d). This biomarker decreased continuously, reaching the lowest measured amount

after 6 months. At the baseline, the levels of HA for P1, P2, and P3 were 168.28, 195.79, and 169.12 ng/ml, respectively. While these values reached 78.91, 85.28, and 81.33, respectively, 6 months after the AD-MSCs injection. Information on other markers including MMP-3, CTX-II, IFN- γ , and TNF- α is available in the supplementary data.

Discussion

Numerous pre-clinical and clinical studies have considered intra-articular MSCs injection to be an efficient treatment for KOA²⁵. That is in spite of the findings of some studies that have shown there is no significant difference in the effectiveness of MSCs compared with the placebo in symptomatic KOA²⁶. Hence, evaluation of the effectiveness of MSCs with

various origins requires further studies. BMSCs and AD-MSCs have been widely used in comparison with the MSCs derived from other origins such as umbilical cord blood²⁷. Harvested MSCs from bone marrow and adipose tissue are similar in phenotype and morphology, and both express surface markers of MSCs such as CD90, CD105, CD44, and CD73²⁸. Moreover, in a clinical trial²⁹ conducted in 2020, no significant difference was reported between autologous AD-MSCs and BMSCs in KOA, and both appeared effective. Nevertheless, AD-MSCs have been more beneficial regarding easier extraction process, longer lasting potential to differentiate, and more potent immunomodulatory effects.

Although a lot of attention has been paid to autologous MSCs, the use of allogeneic MSCs is increasing due to their undeniable merits. Allogeneic MSCs, with similar therapeutic properties to autologous MSCs, would be less costly and immediately available. An additional benefit is for older patients whose MSCs are known to have lower proliferation rates compared to MSCs from younger donors³⁰. Despite concerns about the rejection of allogeneic MSCs by the host's body, the immunomodulatory properties of MSCs cause downregulation of the host's immune responses, which results in inhibition of severe reactions of the host's immune system³¹. In a relatable manner, Vega *et al.*³² investigated the safety and efficacy of allogeneic bone marrow MSCs in KOA. They divided 30 patients into two groups, 15 patients in the test group received 40×10^6 cells and 15 other patients in the control group received hyaluronic acid in the same volume. The results showed allogeneic bone marrow MSCs to be safe and effective.

In the current study, the safety and efficacy of allogeneic AD-MSCs in KOA are described. This pilot

Table 4. Magnetic resonance imaging (MRI) findings.

Patients	Time	MFCC Thickness (mm)	LFCC Thickness (mm)	LTC Thickness (mm)	MTC Thickness (mm)	MFCC Volume (mm ³)	LFCC Volume (mm ³)	LTC Volume (mm ³)	MTC Volume (mm ³)	Joint Effusion	Subchondral Edema
P1	Baseline	0.1	1.71	1.98	0	26	498	465	46	Moderate	Mild subchondral in medial femoral condyle and tibia plateau
	6 months	0	1.1	0.85	0	0	608	440	0	Moderate	Mild subchondral edema in medial tibial plateau
P2	Baseline	1.7	1.45	2.29	2.29	878.8	1594	1576	834.1	Moderate (large barker)	No
	6 months	2.29	2.55	2.8	2.27	1060	1807	1650	980	Moderate (small barker)	No
P3	Baseline	0.78	1.2	1.83	1.59	556	255	334	328	Mild	No
	6 months	0.81	1.5	2.13	2.08	573	328	330	300	No	No

MFCC, medial femoral condyle cartilage; LFCC, lateral femoral condyle cartilage; LTC, lateral tibial cartilage; MTC, medial tibial cartilage; MFCC, medial femoral condyle cartilage; LFCC, lateral femoral condyle cartilage; LTC, lateral tibial cartilage.

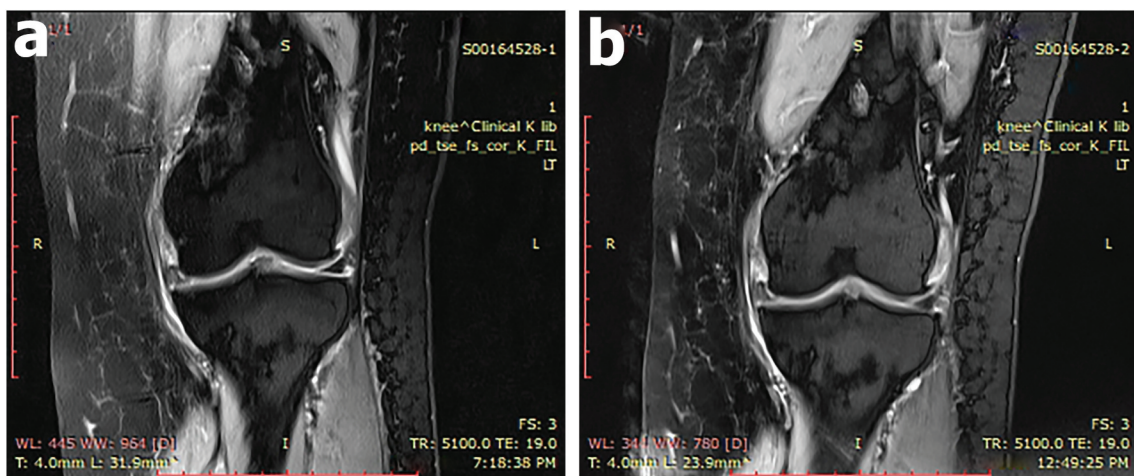


Figure 4. MRI analysis of P2. a: MRI coronal view before AD-MSCs therapy and b: MRI coronal view 6 months after AD-MSCs injection. MRI, magnetic resonance imaging; AD-MSCs, adipose derived mesenchymal stromal cells.

study was conducted in three KOA patients with K/L grade of II/III. Intra-articular injection of 100×10^6 allogeneic AD-MSCs did not cause any SAEs and only one patient experienced mild pain and swelling 1 week after the injection. These AEs have been reported in other studies that used autologous or allogeneic MSCs in KOA^{33,34}. For instance, Pers *et al.*³⁵ in a phase I study evaluated the safety of autologous AD-MSCs in 18 patients with KOA and reported transient pain and swelling in the knee joints of four patients after injection.

The allogeneic AD-MSCs generally improved all clinical outcomes including VAS, WOMAC, KOOS, and SF-36 scores at 6-months follow-up. Notable pain improvements were observed in VAS and WOMAC assessments after 3 months. A decreasing trend in VAS and WOMAC scores continuing until 6 months after the injection confirmed the improvement process. Moreover, the findings of KOOS showed remarkable amelioration of pain, symptoms, quality of life, daily function, and recreation and sport status for all patients. Measuring KOOS, WOMAC, and VAS together is common for accurate monitoring of MSCs injection results in KOA. A similar clinical trial³⁶ of autologous AD-MSCs injection demonstrated improvements in VAS, KOOS, and WOMAC assessments as well. Enhancement in the health status of our patients was noted via SF-36 questionnaire. Based on SF-36 analysis, the patients' overall mental and physical health improved after the 6-months follow-up.

In addition to the positive effects on clinical conditions, comparison of the baseline and endpoint MRI revealed no progression of KOA. Analysis of medial

femoral condyle cartilage (MFCC), lateral femoral condyle cartilage (LFCC), lateral tibial cartilage (LTC), and medial tibial cartilage (MTC) in P2 and P3 showed a slight improvement. Nevertheless, a comparison of MRI findings before and 6 months after injection into P1 shows a slight reduction in cartilage volume and thickness, indicating slower progression of osteoarthritis. Due to the fact that OA is a progressive degenerative disease, slowing the progression of the disease could be beneficial for the patient. The effectiveness of MSCs in preventing KOA progression was reported in this study is in consistence with the results of a published study by Sekiya *et al.*³⁷ in 2021. These alterations after AD-MSCs therapy indicate the possible differentiation of AD-MSCs into chondrocytes and extra cellular matrix synthesis. However, no significant changes were reported in subchondral edema and joint effusion. Nonetheless, since no severe joint effusion and subchondral edema were reported in any of the patients at baseline, the role of AD-MSCs on healing effusion and subchondral edema is not clarified in this study and this issue should be investigated in more severe cases. According to similar studies, the effectiveness of MSCs in cartilage production depends on the dose of injected cells. For instance, MRI findings of a study³⁸ that investigated three different doses of autologous AD-MSCs in KOA demonstrated that in the high-dose group (100×10^6 cells), the size of cartilage defect decreased dramatically.

In this study, we analyzed the levels of some biomarkers to carefully examine the effects of AD-MSCs on reducing inflammation and cartilage damage. Investigation of serum inflammatory markers has

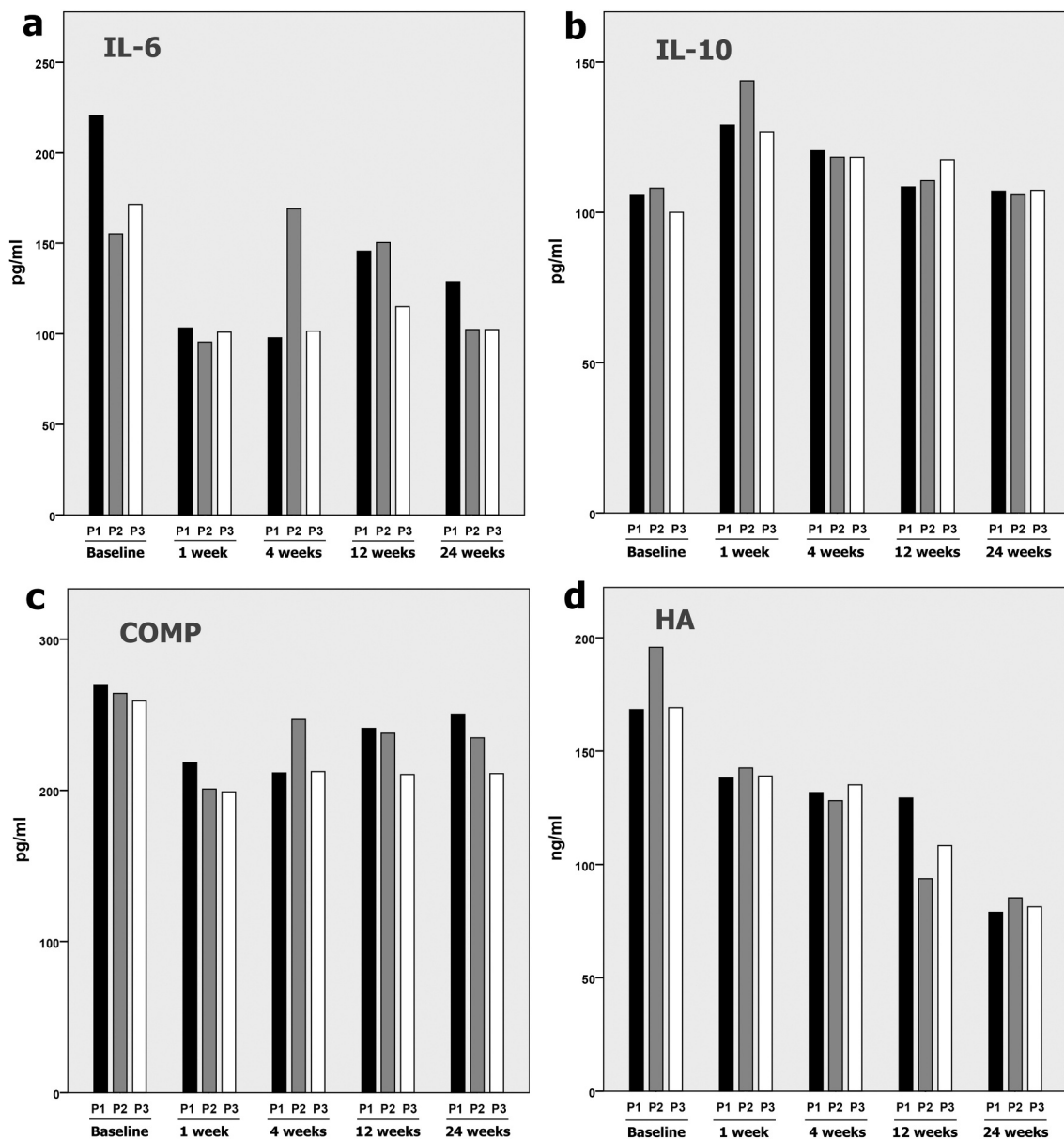


Figure 5. Changes in the expression of biomarkers in the serum of the patients. (a): IL-6, (b): IL-10, (c): COMP, (d): HA. IL-6, interleukin-6; IL-10, interleukin-10; COMP, cartilage oligomeric matrix protein; HA, hyaluronic acid; AD-MSCs, adipose derived mesenchymal stromal cells.

been performed in similar studies in recent years because some inflammatory cytokines such as IL-6 can play a role in KOA progress³⁹. In this regard, Li *et al.*⁴⁰ evaluated the levels of serum IL-6 and TNF- α in patients after injecting autologous BMSCs. Serum levels of these markers decreased significantly after 6 and 12 months in comparison with the baseline. The efficacy of AD-MSCs in KOA is quite consistent with the dramatic decline in the levels of these markers 1 week after AD-MSCs injection. During the next follow-ups, the expression of IL-6 increased slightly and then remained constant. This slight increase after 4 weeks

may be due to the differentiation of AD-MSCs into chondrocytes. IL-6 is an impactful pro-inflammatory cytokine, which its elevated concentrations have been reported in various inflammatory diseases, including rheumatoid arthritis as well as coronavirus disease 2019 (COVID-19)⁴¹. Furthermore, IL-10 was another inflammatory marker assessed in this study. Unlike IL-6, AD-MSCs application increased the levels of IL-10 as a result of a reduction in proliferation of T cells⁴². Therefore, the improvement in KOA was remarkable 1 week after injection of AD-MSCs. COMP and HA are two other markers that were examined to measure the

effectiveness of AD-MSCs in preventing cartilage damage. The amounts of COMP and HA are directly related to cartilage damage⁴³. After AD-MSCs therapy, a noticeable decrease in their levels was observed. This decreasing trend continued for HA until the 6-months follow-up while regarding COMP level, a slight elevation took place 4 weeks after treatment that stayed almost the same in 3 and 6-months follow-ups. In a similar study, Chahal *et al.*⁴⁴ evaluated the level of serum, plasma, and urine biomarkers following the treatment with autologous BM-MSCs injection in KOA. COMP and HA were two biomarkers that their levels in plasma remained approximately constant during the 12 month follow-up. Other cytokines such as CTX-II and MMP-3, which have been suggested as indicators of KOA⁴³, and two other inflammatory markers (IFN- γ and TNF- α) were tested in this study, but no significant changes were noted. The level of these biomarkers did not follow a similar trend in patients after cell therapy and needs further investigation and more patients.

In addition to the limited number of patients, the lack of a control group was one of the major limitations of this study that should be addressed in future studies. Placebo controls have been evidenced to be essential in evaluating the efficacy of intra-articular treatments in OA studies⁴⁵.

Conclusion

This phase I study presents that intra-articular injection of allogeneic AD-MSCs was safe and induced reduction of cartilage damage in KOA. Although this was a phase I study in a limited number of patients and with a relatively short follow-up period, it showed that AD-MSCs transplantation was safe and well tolerated in patients with KOA and provided encouraging preliminary evidence of efficacy and improvement in numerous secondary endpoints. Long-term follow-up, larger and controlled studies are necessary to confirm whether AD-MSCs injection can improve signs and symptoms in KOA.

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Authors' contributions

B.S., S.N., M.M., S.K.T., M.H., and M.G.L. collected data. B. S. drafted the manuscript. A.T. and M.V. reviewed the manuscript and checked the scientific content. A.B., H.M., and M. B. performed critical reviewing of the manuscript. A.B. M. performed language polishing. M.M. and N.N. analyzed MRI findings. A.B.F., J.M., and M.V. developed the concept and outlines, and confirmed the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

The study included only adults and written informed consent have been obtained from the patients. All procedures were conducted in accordance with the relevant approved regulations, guidelines, and the Declaration of Helsinki. This study was approved by the Ethics Committee of the Royan Institute (IR.ACECR.ROYAN.REC.1396.138) and registered in the Iranian Registry of Clinical Trials (<https://www.irct.ir>) with identifier IRCT20080728001031N23.

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References

1. Testa G, Giardina SMC, Culmone A, Vescio A, Turchetta M, Cannavò S Pavone V. Intra-Articular injections in knee osteoarthritis: a review of literature. *J Funct Morphol Kinesiol*. 2021;6(1): 15. doi:10.3390/jfkm6010015.

2. Shah K, Zhao AG Sumer H. New approaches to treat osteoarthritis with mesenchymal stem cells. *Stem Cells Int.* 2018;2018: 1–9. doi:10.1155/2018/5373294.
3. Deshpande BR, Katz JN, Solomon DH, Yelin EH, Hunter DJ, Messier SP, Suter LG Losina E. Number of persons with symptomatic knee osteoarthritis in the US: impact of race and ethnicity, age, sex, and obesity. *Arthritis Care Res.* 2016;68(12): 1743–1750. doi:10.1002/acr.22897.
4. Sasaki E, Ota S, Chiba D, Kimura Y, Sasaki S, Yamamoto Y, Tsuda E, Nakaji S Ishibashi Y. Early knee osteoarthritis prevalence is highest among middle-aged adult females with obesity based on new set of diagnostic criteria from a large sample cohort study in the Japanese general population. *Knee Surg Sports Traumatol Arthrosc.* 2020;28(3): 984–994. doi:10.1007/s00167-019-05614-z.
5. Driban JB, Harkey MS, Barbe MF, Ward RJ, MacKay JW, Davis JE, Lu B, Price LL, Eaton CB Lo GH, McAlindon TE. Risk factors and the natural history of accelerated knee osteoarthritis: a narrative review. *BMC Musculoskelet Disord.* 2020;21(1): 1–11. doi:10.1186/s12891-020-03367-2.
6. Lu Y, Zhang W, Wang J, Yang G, Yin S, Tang T, Yu C Jiang X. Recent advances in cell sheet technology for bone and cartilage regeneration: from preparation to application. *Int J Oral Sci.* 2019;11(2): 1–13. doi:10.1038/s41368-019-0050-5.
7. Zhou Z, Wu W, Fang J Yin J. Polymer-Based porous microcarriers as cell delivery systems for applications in bone and cartilage tissue engineering. *Int Mater Rev.* 2021;66(2): 77–113. doi:10.1080/09506608.2020.1724705.
8. Rahmani Del Bakhshayesh A, Babaie S, Tayefi Nasrabadi H, Asadi N, Akbarzadeh A Abedelahi A. An overview of various treatment strategies, especially tissue engineering for damaged articular cartilage. *Artif Cells Nanomed Biotechnol.* 2020;48(1): 1089–1104. doi:10.1080/21691401.2020.1809439.
9. Urlić I Ivković A. Cell sources for cartilage repair—biological and clinical perspective. *Cells.* 2021;10(9): 2496. doi:10.3390/cells10092496.
10. Musiał-Wysocka A, Kot M Majka M. The pros and cons of mesenchymal stem cell-based therapies. *Cell Transplant.* 2019;28(7): 801–812. doi:10.1177/0963689719837897.
11. Macrin D, Joseph JP, Pillai AA Devi A. Eminent sources of adult mesenchymal stem cells and their therapeutic imminence. *Stem Cell Rev Rep.* 2017;13(6): 741–756. doi:10.1007/s12015-017-9759-8.
12. Dabrowska S, Andrzejewska A, Janowski M Lukomska B. Immunomodulatory and regenerative effects of mesenchymal stem cells and extracellular vesicles: therapeutic outlook for inflammatory and degenerative diseases. *Front Immunol.* 2021;11: 3809. doi:10.3389/fimmu.2020.591065.
13. Vasiliadis AV Galanis N. Effectiveness of ad-MSCs injections for the treatment of knee osteoarthritis: analysis of the current literature. *J Stem Cells Regen Med.* 2020;16(1): 3.
14. Lam AT, Reuveny S Oh SK. Human mesenchymal stem cell therapy for cartilage repair: review on isolation, expansion, and constructs. *Stem Cell Res.* 2020;44(101738): 101738. doi:10.1016/j.scr.2020.101738.
15. McIntyre JA, Jones IA, Han B Vangsness CT. Intra-Articular mesenchymal stem cell therapy for the human joint: a systematic review. *Am J Sports Med.* 2018;46(14): 3550–3563. doi:10.1177/0363546517735844.
16. Burrow KL, Hoyland JA Richardson SM. Human adipose-derived stem cells exhibit enhanced proliferative capacity and retain multipotency longer than donor-matched bone marrow mesenchymal stem cells during expansion in vitro. *Stem Cells Int.* 2017;2017: 1–15. doi:10.1155/2017/2541275.
17. Han X, Yang B, Zou F Sun J. Clinical therapeutic efficacy of mesenchymal stem cells derived from adipose or bone marrow for knee osteoarthritis: a meta-analysis of randomized controlled trials. *J Comp Eff Res.* 2020;9(5): 361–374. doi:10.2217/ce-2019-0187.
18. Pers Y-M, Quentin J, Feirreira R, Espinoza F, Abdellaoui N, Erkilic N, Cren M, Dufourcq-Lopez E, Pullig O, Nöth U, Jorgensen C Louis-Plence P. Injection of adipose-derived stromal cells in the knee of patients with severe osteoarthritis has a systemic effect and promotes an anti-inflammatory phenotype of circulating immune cells. *Theranostics.* 2018;8(20): 5519. doi:10.7150/thno.27674.
19. Wang Y, Shimmin A, Ghosh P, Marks P, Linklater J, Connell D, Hall S, Skerrett D, Itescu S Cicuttini FM. Safety, tolerability, clinical, and joint structural outcomes of a single intra-articular injection of allogeneic mesenchymal precursor cells in patients following anterior cruciate ligament reconstruction: a controlled double-blind randomised trial. *Arthritis Res Ther.* 2017;19(1): 180. doi:10.1186/s13075-017-1391-0.
20. Park YB, Ha CW, Lee CH, Yoon YC Park YG. Cartilage regeneration in osteoarthritic patients by a composite of allogeneic umbilical cord blood-derived mesenchymal stem cells and hyaluronate hydrogel: results from a clinical trial for safety and proof-of-concept with 7 years of extended follow-up. *Stem Cells Trans Med.* 2017;6(2): 613–621. doi:10.5966/sctm.2016-0157.
21. Soltani SK, Forogh B, Ahmadbeigi N, Kharazi HH, Fallahzadeh K, Kashani L, Karami M, Kheyrollah Y Vasei M. Safety and efficacy of allogenic placental mesenchymal stem cells for treating knee osteoarthritis: a pilot study. *Cytotherapy.* 2019;21(1): 54–63. doi:10.1016/j.jcyt.2018.11.003.
22. Zhao X, Ruan J, Tang H, Li J, Shi Y, Li M, Li S, Xu C, Lu Q Dai C. Multi-Compositional MRI evaluation of repair cartilage in knee osteoarthritis with treatment of allogeneic human adipose-derived mesenchymal progenitor cells. *Stem Cell Res Ther.* 2019;10(1): 308. doi:10.1186/s13287-019-1406-7.
23. Lu L, Dai C, Du H, Li S, Ye P, Zhang L, Wang X, Song Y, Togashi R Vangsness CT, Bao C. Intra-Articular injections of allogeneic human adipose-derived mesenchymal progenitor cells in patients with symptomatic bilateral knee osteoarthritis:

- a phase I pilot study. *Regen Med.* 2020;15(5): 1625–1636. doi:10.2217/rme-2019-0106.
24. Sadri B, Sh N, Hossein-Khannazer N, Mohammadi J Vosough M. Current and novel theranostic modalities for knee osteoarthritis. *Сеченовский Вестник.* 2021;12 (3): 17–30.
25. Ha C-W, Park Y-B, Kim SH Lee HJ. Intra-Articular mesenchymal stem cells in osteoarthritis of the knee: a systematic review of clinical outcomes and evidence of cartilage repair. *Arthroscopy.* 2019;35(1): 277–288. doi:10.1016/j.arthro.2018.07.028.
26. Dai W, Leng X, Wang J, Shi Z, Cheng J, Hu X Ao Y. Intra-Articular mesenchymal stromal cell injections are no different from placebo in the treatment of knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. *Arthroscopic.* 2021;37 (1): 340–358. doi:10.1016/j.arthro.2020.10.016.
27. Xiang X-N, Zhu S-Y, H-C H, Yu X, Xu Y He CQ. Mesenchymal stromal cell-based therapy for cartilage regeneration in knee osteoarthritis. *Stem Cell Res Ther.* 2022;13(1): 1–20. doi:10.1186/s13287-021-02689-9.
28. Alonso-Goulart V, Ferreira LB, Duarte CA, de Lima IL, Ferreira ER, de Oliveira BC, Vargas LN, de Moraes DD, Silva IBB de Oliveira Faria R, Souza AGD, Castro-Filice LDS. Mesenchymal stem cells from human adipose tissue and bone repair: a literature review. *Biotechnol Res Innov.* 2018;2(1): 74–80. doi:10.1016/j.biori.2017.10.005.
29. Estrada E, Décima JL, Rodríguez M, Di Tomaso M Roberti J. Patient-Reported outcomes after platelet-rich plasma, bone marrow aspirate, and adipose-derived mesenchymal stem cell injections for symptomatic knee osteoarthritis. *Clin Med Insights Arthritis Musculoskelet Disord.* 2020;13: 1179544120931086. doi:10.1177/1179544120931086.
30. Mautner K, Carr D, Whitley J Bowers R. Allogeneic versus autologous injectable mesenchymal stem cells for knee osteoarthritis: Review and current status. *Tech Orthop.* 2019;34(4): 244–256. doi:10.1097/BTO.0000000000000357.
31. Weiss ARR Dahlke MH Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead mscs. *Front immunol.* 2019;10: 1191.
32. Vega A, Martín-Ferrero MA, Del Canto F, Alberca M, García V, Munar A, Orozco L, Soler R, Fuertes JJ, Huguet M, Sánchez A García-Sancho J. Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells: a randomized controlled trial. *Transplantation.* 2015;99(8): 1681–1690. doi:10.1097/TP.0000000000000678.
33. Song Y, Du H, Dai C, Zhang L, Li S, Hunter DJ, Lu L Bao C. Human adipose-derived mesenchymal stem cells for osteoarthritis: a pilot study with long-term follow-up and repeated injections. *Regen Med.* 2018;13(3): 295–307. doi:10.2217/rme-2017-0152.
34. Cabon Q, Febre M, Gomez N, Cachon T, Pillard P, Carozzo C, Saulnier N, Robert C, Livet V Rakic R, Plantier N, Saas P, Maddens S, Viguier E. Long-Term safety and efficacy of single or repeated intra-articular injection of allogeneic neonatal mesenchymal stromal cells for managing pain and lameness in moderate to severe canine osteoarthritis without anti-inflammatory pharmacological support: pilot clinical study. *Front Vet Sci.* 2019;6:10.
35. Pers Y-M, Rackwitz L, Ferreira R, Pullig O, Delfour C, Barry F, Sensebe L, Casteilla L, Fleury S, Bourin P, Noël D, Canovas F, Cyteval C, Lisignoli G, Schrauth J, Haddad D, Domergue S, Noeth U Jorgensen C. Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial. *Stem Cells Trans Med.* 2016;5(7): 847–856. doi:10.5966/sctm.2015-0245.
36. Jo CH, Chai JW, Jeong EC, Oh S, Shin JS, Shim H Yoon KS. Intra-Articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a 2-year follow-up study. *Am J Sports Med.* 2017;45(12): 2774–2783. doi:10.1177/0363546517716641.
37. Sekiya I, Katano H, Mizuno M, Koga H, Masumoto J, Tomita M Ozeki N. Alterations in cartilage quantification before and after injections of mesenchymal stem cells into osteoarthritic knees. *Sci Rep.* 2021;11(1): 1–14. doi:10.1038/s41598-021-93462-8.
38. Jo CH, Lee YG, Shin WH, Kim H, Chai JW, Jeong EC, Kim JE, Shim H, Shin JS Shin IS, Ra JC, Oh S, Yoon KS. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *Stem Cells.* 2014;32(5): 1254–1266. doi:10.1002/stem.1634.
39. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J Ding C. Circulating levels of il-6 and tnf- α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Ostenarthr Cartil.* 2010;18 (11): 1441–1447. doi:10.1016/j.joca.2010.08.016.
40. Li J, Shao Q, Zhu X Sun G. Efficacy of autologous bone marrow mesenchymal stem cells in the treatment of knee osteoarthritis and their effects on the expression of serum tnf- α and il-6. *J Musculoskelet Neuronal Interact.* 2020;20 (1): 128.
41. Potere N, Batticciotto A, Vecchié A, Porreca E, Cappelli A, Abbate A, Dentali F Bonaventura A. The role of il-6 and il-6 blockade in COVID-19. *Expert Rev Clin Immunol.* 2021;17(6): 601–618. doi:10.1080/1744666X.2021.1919086.
42. Haddad R Saldanha-Araujo F. Mechanisms of t-cell immunosuppression by mesenchymal stromal cells: what do we know so far? *Biomed Res Int.* 2014;2014: 1–14. doi:10.1155/2014/216806.
43. Jiao Q, Wei L, Chen C, Li P, Wang X, Li Y, Guo L, Zhang C Wei X. Cartilage oligomeric matrix protein and hyaluronic acid are sensitive serum biomarkers for early cartilage lesions in the knee joint. *Biomarkers.* 2016;21(2): 146–151. doi:10.3109/1354750X.2015.1118547.
44. Chahal J, Gómez-Aristizábal A, Shestopaloff K, Bhatt S, Chaboureaud A, Fazio A, Chisholm J, Weston A, Chiovitti J Keating A, Kapoor M, Ogilvie-Harris DJ, Syed KA, Gandhi R, Mahomed NN, Marshall KW, Sussman MS, Naraghi AM, Viswanathan S. Bone marrow mesenchymal stromal cell treatment in patients with osteoarthritis results in overall improvement in pain and symptoms and reduces synovial inflammation. *Stem Cells Transl Med.* 2019;8(8): 746–757. doi:10.1002/sctm.18-0183.
45. Bannuru RR, McAlindon TE, Sullivan MC, Wong JB, Kent DM Schmid CH. Effectiveness and implications of alternative placebo treatments: a systematic review and network meta-analysis of osteoarthritis trials. *Ann Intern Med.* 2015;163(5): 365–372. doi:10.7326/M15-0623.