Mesenchymal Stem Cell Implantation in Knee Osteoarthritis

An Assessment of the Factors Influencing Clinical Outcomes

Yong Sang Kim,* MD, Yun Jin Choi,* MD, and Yong Gon Koh,*† MD
Investigation performed at the Center for Stem Cell & Arthritis Research, Department of Orthopaedic Surgery, Yonsei Sarang Hospital, Seoul, Korea

Background: Several clinical studies have reported on cell-based treatment using mesenchymal stem cells (MSCs) for cartilage regeneration in knee osteoarthritis (OA). However, little is known about the factors that influence the clinical outcomes after surgery.

Purpose/Hypothesis: This study aimed to investigate the clinical outcomes of MSC implantation in patients with knee OA and assess the factors that are associated with clinical outcomes. The hypothesis was that factors may exist that could influence clinical outcomes.

Study Design: Case series; Level of evidence, 4.

Methods: A total of 49 patients (55 knees) were retrospectively evaluated after MSC implantation for knee OA. The inclusion criteria were patients who had an isolated full-thickness cartilage lesion and Kellgren-Lawrence OA grade 1 or 2. Clinical outcomes were measured with the International Knee Documentation Committee (IKDC) score, Tegner activity score, and patients' overall satisfaction with the surgery. Statistical analyses were performed to determine the effect of different factors on the clinical outcome.

Results: The mean pre- and postoperative IKDC and Tegner activity scores significantly improved from 37.7 $\pm$ 6.3 to 67.3 $\pm$ 9.5 (IKDC) and from 2.2 $\pm$ 0.7 to 3.8 $\pm$ 0.7 (Tegner) ($P < .001$ for both). Twenty-four patients reported their overall satisfaction with the surgery as excellent (43.6%), 17 as good (30.9%), 11 as fair (20.0%), and 3 as poor (5.5%). There were significant differences in clinical outcomes at the final follow-up among the age and lesion size groups ($P < .05$ for all). Multivariate analyses showed high prognostic significance related to patient age and lesion size, and scatter plots suggested a cutoff age of 60 years and a cutoff lesion size of 6.0 cm^2 for the optimum identification of poor clinical outcomes ($P < .05$ for both).

Conclusion: The clinical outcomes of MSC implantation for knee OA are encouraging. Patient age and lesion size are important factors that affect clinical outcomes; thus, these may serve as a basis for preoperative surgical decisions. Cutoff points exist for the risk of clinical failure in patients older than 60 years and those with a lesion size larger than 6.0 cm^2.

Keywords: mesenchymal stem cell; implantation; prognostic factors; osteoarthritis; knee

Osteoarthritis (OA) is a highly prevalent, progressive, painful joint disease that is accompanied by an increasing deficiency in joint function.[33] The onset of OA is mainly characterized by the gradual loss of articular cartilage due to impaired anabolic and/or catabolic balance, which further affects all the other joint tissues.[17,33] The articular cartilage exhibits little or no ability for self-repair, resulting in progressive tissue loss and dysfunction after isolated cartilage lesions, and this lack of effective repair contributes to the widespread degeneration of the joint affected by OA.[14] Although joint replacement with artificial components have been suggested as the definitive treatment for end-stage OA, the limited life span of these prostheses may restrict their ability in meeting the demands of younger and more active patients.[31] Furthermore, patients with focal cartilage lesions in the earlier stages of OA remain a challenge because of a combination of high functional demand and limited indications for joint replacement,[34] and restoration of diseased articular cartilage should be the challenge of researchers and clinicians.[36] Therefore, for these patients, significant needs have arisen for the

†Address correspondence to Yong Gon Koh, MD, Department of Orthopaedic Surgery, Yonsei Sarang Hospital, 478-3, Bangbae-dong, Seocho-gu, Seoul, Korea (email: yonggonkoh@gmail.com).
*Center for Stem Cell & Arthritis Research, Department of Orthopaedic Surgery, Yonsei Sarang Hospital, Seoul, Korea.
The authors declared that they have no conflicts of interest in the authorship and publication of this contribution.

The American Journal of Sports Medicine, Vol. 43, No. 9
DOI: 10.1177/0363546515588317

development of novel therapy to protect the cartilage, inhibit further loss, and even reestablish the knee's structural integrity.[12]

As a potential cell-based therapy for cartilage repair, mesenchymal stem cells (MSCs) have been suggested for treating diseased articular cartilage because of their ability to differentiate into chondrocytes and their paracrine effects of secreted bioactive materials.[4,8,37] Currently, several clinical studies concerning the use of MSCs as a cell-based treatment for knee OA have reported encouraging outcomes after MSC-based treatment for knee OA.[21,26,28-30,38] However, to date, no studies have assessed the factors that influence the outcomes of MSC-based treatment for knee OA. The identification of factors associated with favorable and unfavorable outcomes would provide patients with accurate expectations of the MSC-based treatment. Accordingly, the aims of this study were to investigate the clinical outcomes of MSC implantation in patients with knee OA and assess the factors that are associated with clinical outcomes. We hypothesized that there are factors that can increase the risk of clinical failure, which can be isolated and verified by performing different statistical analyses.

METHODS

Patient Enrollment

We retrospectively reviewed the medical records of 62 consecutive patients (70 knees) with cartilage lesions in the knees who were treated with arthroscopic MSC implantation with fibrin glue as a scaffold for cartilage regeneration between November 2011 and December 2012. The study protocol was approved by our hospital's institutional review board, and all patients provided written informed consent before treatment. The inclusion criteria, determined by medical records, plain radiographs, and magnetic resonance imaging (MRI), were patients who had an isolated full-thickness cartilage lesion and Kellgren-Lawrence[24] OA grade 1 or 2 with symptoms of knee joint pain and/or functional limitations despite a minimum of 3 months of nonsurgical treatments. The exclusion criteria were the patients with multiple cartilage lesions in their knees, previous surgical treatment, knee instability, varus or valgus malalignment of $\geq 5^\circ$ of the knee joint, metabolic arthritis, joint infections, or large meniscal tears. Of the 62 patients (70 knees), 49 patients (55 knees) met the inclusion criteria and were ultimately included. The study population included 26 men and 29 women, with a mean age of 58.1 years (range, 48-69 years). The average preoperative body mass index (BMI) was 26.5 kg/m^2 (range, 19.2-31.2 kg/m^2), and the mean follow-up period was 26.7 months (range, 24-36 months) (Table 1).

TABLE 1
Demographic Data[a]

Age, y	58.1 ± 6.0 (48-69)
Sex, male/female, n	26/29
Side of involvement, right/left/both, n	24/25/3
Body mass index, kg/m^2	26.5 ± 3.2 (19.2-31.2)
Follow-up period, mo	26.7 ± 3.6 (24-36)
Lesion size, cm^2	5.7 ± 2.4 (2.3-12.4).
Lesion location, medial femoral condyle/lateral femoral condyle/trochlea, n	29/17/9

[a]Values are expressed as mean ± SD (range) unless otherwise indicated.

MSC Preparation and Surgical Procedures

Sample collections and MSC isolation were performed as described previously.[26,28] In brief, 1 day before arthroscopic surgery, adipose tissue was harvested from the patients' buttocks through tumescent liposuction. After isolating and characterizing the adipose-derived cells as described previously,[26,28,33] we confirmed that the adipose-derived cells contained MSCs. After isolation, the adipose-derived stem cells represented a mean of 9.4% of the stromal vascular fraction cells (range, 8.4%-10.3% of the stromal vascular fraction cells). After the stromal vascular fractions were isolated, a mean of 4.3×10^6 stem cells (9.4% of 4.6×10^7 stromal vascular fraction cells; range, 3.7-4.8 $\times$ 10^6) were prepared. Accordingly, we used an average of 4.6×10^7 stromal vascular fraction cells, which contained an average of 4.3×10^6 stem cells for MSC implantation. The surgical procedures were identical in all the patients, and they were performed as described previously.[26] Before MSC implantation, accurate debridement of all unstable and damaged cartilage in the lesion was performed. The prepared MSCs were loaded into the fibrin glue product (Greenplast kit; Greencross), which was used as a scaffold for MSC implantation. Next, after the arthroscopic fluid was extracted, the MSCs mixed with fibrin glue were implanted into the lesion site under arthroscopic guidance. Then, the applied MSCs mixed with fibrin glue were manipulated using the probe to evenly cover the surface of cartilage lesion. No marrow-stimulation procedures such as microfracture surgery, subchondral drilling, or abrasion arthroplasty were performed before this procedure. After performing the arthroscopic procedure, the knee was immobilized for 2 weeks with a knee brace, and after the sutures were removed, the patients began range of motion exercises, including both active and passive exercises of the knee joint. Partial weightbearing was initiated at 2 weeks after arthroscopy, and full weightbearing was permitted at 4 weeks postoperatively. Sports and high-impact activities were allowed after 3 months, and the full return to normal sports or recreational activities was allowed according to patients' individual recovery.

Outcome Assessment

All patients were evaluated clinically preoperatively and during follow-up. For the clinical evaluation, the International Knee Documentation Committee (IKDC)[20] and the Tegner activity scale[43] were used to determine joint

TABLE 2
Clinical Outcomes by Age[a]

	Age, y			
	<50 (n = 6)	50-59 (n = 23)	≥60 (n = 26)	*P* Value
IKDC score				
Preoperative	40.7 ± 3.6	38.7 ± 7.4	36.1 ± 5.4	.218[b]
Final follow-up	72.8 ± 10.3	70.6 ± 7.9	63.1 ± 9.1	**.004**[b]
Tegner activity scale				
Preoperative	2.3 ± 0.5	2.1 ± 0.8	2.2 ± 0.6	.834[b]
Final follow-up	4.0 ± 0.6	4.0 ± 0.5	3.5 ± 0.7	**.008**[b]
Patients' overall satisfaction, n (%)				**<.001**[c]
Excellent	5 (83.3)	15 (65.2)	4 (15.4)	
Good	1 (16.7)	5 (21.7)	11 (42.3)	
Fair	0 (0)	3 (13.1)	8 (30.8)	
Poor	0 (0)	0 (0)	3 (11.5)	

[a]Values are expressed as mean ± SD unless otherwise indicated. Boldface indicates statistical significance ($P < .05$). IKDC, International knee Documentation Committee.
[b]Kruskal-Wallis test.
[c]Fisher exact test.

function and sports activities. Additionally, patients rated their overall satisfaction with the operation as *excellent, good, fair*, or *poor*. To assess patient characteristics (age, sex, side of involvement, and BMI) and cartilage lesion variables (size and location) that may influence clinical outcomes, the factors were divided into subgroups. We divided the patients according to age (<50, 50-59, and ≥60 years), sex, side of involvement (right and left), BMI (<20.0, 20.0-24.9, 25-29.9, and ≥30.0 kg/m^2), lesion size (<3.0, 3.0-5.9, 6.0-8.9, and ≥9.0 cm^2), and lesion location (medial femoral condyle, lateral femoral condyle, and trochlea).

Statistical Analysis

The principal dependent variables were the IKDC score, Tegner activity score, and patients' overall satisfaction with the operation at the final follow-up. Descriptive statistics were calculated as means ± SDs. The Wilcoxon signed-rank test was used to evaluate differences between the preoperative and final follow-up values. A Fisher exact test was used to compare the categorical data. The associations among factors were also examined on the basis of the clinical outcomes. Differences between the groups were analyzed using the Mann-Whitney *U* test or the Kruskal-Wallis test for multiple comparisons. The Spearman rank-order correlation test was used to evaluate the potential bivariate associations between the different factors to test whether there was a statistically significant correlation. Bivariate and multivariate logistic regression analyses were used to assess the various factors that were independently associated with clinical failure. All multivariate models were controlled for differences in age, sex, side of involvement, BMI, and lesion size and location. For the logistic regression models, we reported odds ratios and 95% CIs relative to a chosen reference group. Statistical analyses were performed using SPSS (v 13.0; IBM Corp), with significance defined as $P < .05$.

RESULTS

Clinical Outcomes

Before MSC implantation, the mean IKDC score was 37.7 ± 6.3, and the mean Tegner activity score was 2.2 ± 0.7. At final follow-up, the mean IKDC and Tegner activity scores significantly improved to 67.3 ± 9.5 and 3.8 ± 0.7, respectively ($P < .001$ for both). With regard to overall satisfaction with the surgery, 24 patients reported their satisfaction as excellent (43.6%), 17 as good (30.9%), 11 as fair (20.0%), and 3 as poor (5.5%).

Association Between Patient Characteristics and Clinical Outcomes

The clinical outcomes preoperatively and at final follow-up in each age group are shown in Table 2. There were significant differences in clinical outcomes at final follow-up among the age groups (IKDC score, $P = .004$; Tegner activity score, $P = .008$; and patients' overall satisfaction, $P < .001$). However, no significant differences were found among the sex, side of involvement, or BMI groups (Tables 3 and 4). According to multivariate analyses, patient age accounted for 16.7% of the variability in the follow-up IKDC score. Interestingly, a cutoff point existed for the prognostic influence of age. As illustrated in the scatter plot (Figure 1A), the IKDC score of patients aged >60 years was significantly more often <67.3 (the mean IKDC score at final follow-up) compared with that of patients aged <60 years. Moreover, only 1 of 6 patients (16.7%) aged <50 years had a Tegner activity score of <4, which was similarly observed in 3 of 23 patients (13%) aged 50 to 59 years; however, many patients had a Tegner activity score of <4 among those aged ≥60 years (57.7%; 15/26) (Figure 1B). Therefore, we considered the age of 60 years as a cutoff value for obtaining encouraging outcomes after MSC implantation.

TABLE 3
Clinical Outcomes by Sex and Side of Involvement[a]

	Sex			Side of Involvement		
	Male (n = 26)	Female (n = 29)	*P* Value	Right (n = 24)	Left (n = 31)	*P* Value
IKDC score						
Preoperative	37.3 ± 6.2	38.0 ± 6.5	.753[b]	36.8 ± 5.8	38.4 ± 6.6	.457[b]
Final follow-up	65.9 ± 9.5	68.6 ± 9.4	.333[b]	68.7 ± 10.5	66.2 ± 8.7	.209[b]
Tegner activity scale						
Preoperative	2.1 ± 0.7	2.3 ± 0.6	.204[b]	2.3 ± 0.7	2.2 ± 0.6	.589[b]
Final follow-up	3.7 ± 0.6	3.8 ± 0.7	.267[b]	3.9 ± 0.7	3.6 ± 0.6	.139[b]
Patients' overall satisfaction, n (%)			.577[c]			.079[c]
Excellent	12 (46.2)	12 (41.4)		14 (58.4)	10 (32.3)	
Good	4 (15.4)	13 (44.8)		5 (20.8)	12 (38.7)	
Fair	9 (34.6)	2 (6.9)		5 (20.8)	6 (19.3)	
Poor	1 (3.8)	2 (6.9)		0 (0)	3 (9.7)	

[a]Values are expressed as mean ± SD unless otherwise indicated. IKDC, International knee Documentation Committee.
[b]Mann-Whitney *U* test.
[c]Fisher exact test.

TABLE 4
Clinical Outcomes by Body Mass Index[a]

	Body Mass Index, kg/m^2				
	<20.0 (n = 4)	20.0-24.9 (n = 18)	25.0-29.9 (n = 25)	≥30.0 (n = 8)	*P* Value
IKDC score					
Preoperative	40.0 ± 4.1	38.9 ± 5.4	37.0 ± 7.2	35.9 ± 6.0	.410[b]
Final follow-up	68.8 ± 9.7	68.2 ± 8.0	66.6 ± 10.0	66.8 ± 12.2	.883[b]
Tegner activity scale					
Preoperative	2.3 ± 0.5	2.4 ± 0.6	2.1 ± 0.7	2.0 ± 0.5	.233[b]
Final follow-up	3.8 ± 0.5	3.7 ± 0.5	3.7 ± 0.8	4.0 ± 0.8	.735[b]
Patients' overall satisfaction, n (%)					.112[c]
Excellent	3 (75)	10 (55.6)	9 (36)	2 (25)	
Good	1 (25)	6 (33.3)	6 (24)	4 (50)	
Fair	0 (0)	2 (11.1)	8 (32)	1 (12.5)	
Poor	0 (0)	0 (0)	2 (8)	1 (12.5)	

[a]Values are expressed as mean ± SD unless otherwise indicated. IKDC, International knee Documentation Committee.
[b]Kruskal-Wallis test.
[c]Fisher exact test.

Association Between Cartilage Lesion Variables and Clinical Outcomes

The mean size of the cartilage lesions was 5.7 ± 2.4 cm^2 (range, 2.3-12.4 cm^2); with regard to lesion location, 29 knees had lesions in the medial femoral condyle, 17 had lesions in the lateral femoral condyle, and 9 had lesions in the trochlea (Table 1). The clinical outcomes preoperatively and at final follow-up in each lesion size group are shown in Table 5. There were significant differences in clinical outcomes at final follow-up among the 4 lesion size groups (IKDC score, $P = .005$; Tegner activity score, $P = .026$; patients' overall satisfaction, $P < .001$). However, no significant difference in the clinical outcomes at final follow-up was found among the location groups (Table 6). According to multivariate analyses, the lesion size accounted for 22% of the variability in the follow-up IKDC score. Interestingly, a cutoff point existed for the prognostic influence of the lesion size. As illustrated in the scatter plot in Figure 1C, the IKDC score of patients with a lesion size >6.0 cm^2 was significantly more often <67.3 (the mean IKDC score at final follow-up) compared with that of patients with a lesion size <6.0 cm^2. Moreover, only 1 of 8 knees (12.5%) with a lesion size <3.0 cm^2 had a Tegner activity score of <4, which was similar for 5 of 24 patients (20.8%) with a lesion size of 3.0 to 5.9 cm^2; however, the percentage of patients with a Tegner activity score of <4 was significantly higher in patients with a lesion size of 6.0 to 8.9 cm^2 (50%; 9/18) and in patients with a lesion size of ≥9.0 cm^2 (80%; 4/5) (Figure 1D). Therefore, we considered a lesion size of 6.0 cm^2 as a cutoff value for obtaining encouraging outcomes after MSC implantation.

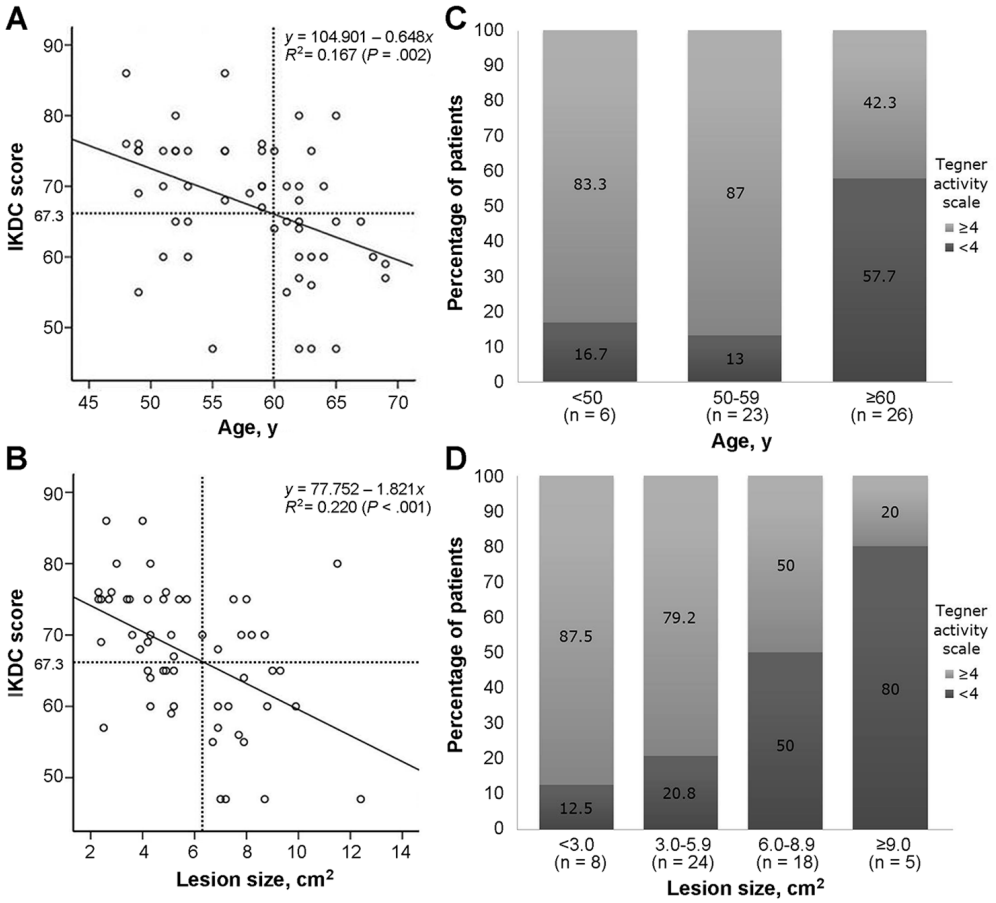

Figure 1. Correlations between International Knee Documentation Committee (IKDC) score at final follow-up and (A) age and (B) lesion size, and percentage of patients in each (C) age group and (D) lesion size group according to Tegner activity score at final follow-up.

Correlations Between the Different Factors and Clinical Outcomes

The associations among the different factors are shown in the Appendix (available in the online version of this article at http://ajsm.sagepub.com/supplemental). The bivariate correlation showed a statistically significant association between age and lesion size (correlation coefficient [CC] = 0.471, $P < .001$) and between BMI and lesion size (CC = 0.408, $P = .002$). There were also significant associations between age and clinical outcomes (IKDC score: CC = –0.443, $P < .001$; Tegner activity score: CC = –0.432, $P < .001$) and between lesion size and clinical outcomes (IKDC score: CC = –0.471, $P < .001$; Tegner activity score: CC = –0.408, $P = .002$). We used logistic regression models to assess the effect of different factors on clinical outcomes. We defined clinical failure as an IKDC score of <68 (mean IKDC score at final follow-up, 67.3), a Tegner activity score of <4 (mean Tegner activity score at final follow-up, 3.8), or fair or poor patient satisfaction at final follow-up. The final model, as shown in Table 7, controlled for age, sex, side of involvement, BMI, and lesion size and location. Age and lesion size were independent predictors of clinical failure after MSC implantation ($P = .003$ and .013, respectively). Compared with patients aged <50 years, those aged 50 to 59 years were 1.39 times more likely to have clinical failure after MSC implantation (95% CI, 0.13-14.78) and those aged >60 years were 9.44 times more likely to have clinical failure (95% CI, 0.95-93.64). Compared with patients with a lesion size <3.0 cm^2, those with a lesion size of 3.0 to 5.9 cm^2 were 2.88 times more likely to have clinical failure (95% CI, 0.30-27.97). Meanwhile, patients with a lesion size of 6.0 to 8.9 cm^2 were 11.00 times more likely to have clinical failure (95% CI, 1.10-109.67), and those with a lesion size of >9.0 cm^2 were 28.00 times more likely to have clinical failure (95% CI, 1.35-580.59). However, sex ($P = .537$), side of involvement ($P = .260$), BMI ($P = .542$), and lesion location ($P = .465$) did not independently predict clinical outcomes after MSC implantation.

DISCUSSION

Although MSC implantation has demonstrated encouraging clinical efficacy for repairing articular cartilage in knee OA,[26,28] we understand little about the contribution that

TABLE 5
Clinical Outcomes by Lesion Size[a]

	Lesion Size, cm^2				
	< 3.0 (n = 8)	3.0-5.9 (n = 24)	6.0-8.9 (n = 18)	≥9.0 (n = 5)	*P* value
IKDC score					
Preoperative	37.8 ± 6.2	39.1 ± 5.8	36.9 ± 6.5	33.6 ± 7.5	.306[b]
Final follow-up	73.6 ± 8.2	70.4 ± 6.9	61.4 ± 9.3	63.4 ± 11.8	**.005**[b]
Tegner activity scale					
Preoperative	2.5 ± 0.5	2.2 ± 0.7	2.2 ± 0.5	1.6 ± 0.5	.126[b]
Final follow-up	4.0 ± 0.5	4.0 ± 0.6	3.5 ± 0.5	3.2 ± 1.1	**.026**[b]
Patients' overall satisfaction, n (%)					**<.001**[c]
Excellent	7 (87.5)	13 (54.2)	3 (16.7)	1 (20)	
Good	1 (12.5)	9 (37.5)	7 (38.9)	0 (0)	
Fair	0 (0)	2 (8.3)	8 (44.4)	1 (20)	
Poor	0 (0)	0 (0)	0 (0)	3 (60)	

[a]Values are expressed as mean ± SD unless otherwise indicated. Boldface indicates statistical significance ($P < .05$). IKDC, International knee Documentation Committee.
[b]Kruskal-Wallis test.
[c]Fisher exact test.

TABLE 6
Clinical Outcomes by Lesion Location[a]

	Lesion Location			
	Medial Femoral Condyle (n = 29)	Lateral Femoral Condyle (n = 17)	Trochlea (n = 9)	*P* Value
IKDC score				
Preoperative	38.6 ± 5.7	36.6 ± 6.6	36.8 ± 7.7	.583[b]
Final follow-up	69.0 ± 8.1	65.3 ± 10.7	65.4 ± 11.1	.405[b]
Tegner activity scale				
Preoperative	2.2 ± 0.7	2.4 ± 0.7	1.9 ± 0.3	.166[b]
Final follow-up	3.8 ± 0.6	3.6 ± 0.7	3.8 ± 0.7	.598[b]
Patients' overall satisfaction, n (%)				.513[c]
Excellent	14 (48.3)	5 (29.4)	5 (55.6)	
Good	9 (31)	7 (41.2)	1 (11.1)	
Fair	4 (13.8)	4 (23.5)	3 (33.3)	
Poor	2 (6.9)	1 (5.9)	0 (0)	

[a]Values are expressed as mean ± SD unless otherwise indicated. IKDC, International knee Documentation Committee.
[b]Kruskal-Wallis test.
[c]Fisher exact test.

known influential preoperative factors have on clinical outcomes after MSC implantation. This is the first study to assess the effect of factors, including patient characteristics (age, sex, the side of involvement, and BMI) and cartilage lesion variables (size and location), on clinical outcomes after MSC implantation by using different statistical analyses. Understanding the factors that are associated with clinical outcomes will help patients with OA have more realistic expectations after undergoing MSC implantation for their knees.

Other than OA itself, patient characteristics may serve as important selection criteria for stem cell–based repair strategies. An increased age is a significant risk factor for OA that may affect the quality of MSCs.[14] Chang et al[9] compared the number and function of MSCs in articular cartilage among human fetuses, healthy adults (aged 28-45 years), and elderly adults (aged 60-75 years), and they found that MSCs accounted for 94.69%, 4.85%, and 6.33% of the cells in the articular cartilage, respectively ($P < .001$). They also reported that a lower chondrogenic differentiation of MSCs derived from elderly patients might be associated with the development of OA.[9] In addition, several studies have described an age-dependent effect on the properties of adipose-derived MSCs.[6,11,15,44] Choudhery et al[11] investigated the expansion and in vitro differentiation potential of adipose-derived MSCs from younger (<30 years), adult (35-50 years), and older (>60 years) individuals, and they found that older MSCs displayed senescent features compared with cells that were isolated from young donors, concomitant with a reduced viability, proliferation, and differentiation potential. Wu et al[44] compared the biologic features of adipose-derived

TABLE 7
Association Between Patient and Lesion Characteristics and Clinical Failure[a]

Factor	n (%)	Clinical Failure, Odds Ratio (95% CI)	*P* Value
Age, y			**.003**
<50	6 (10.9)	1.00	
50-59	23 (41.8)	1.39 (0.13-14.78)	
≥60	26 (47.3)	9.44 (0.95-93.64)	
Sex			.537
Male	26 (47.3)	1.00	
Female	29 (52.7)	0.71(0.24-2.09)	
Side of involvement			.260
Right	24 (43.6)	1.00	
Left	31 (56.4)	1.88 (0.62-5.65)	
Body mass index, kg/m^2			.542
<20.0	4 (7.3)	1.00	
20.0-24.9	18 (32.7)	1.50 (0.13-17.67)	
25.0-29.9	25 (45.5)	3.25 (0.30-35.66)	
≥30.0	8 (14.5)	1.80 (0.12-26.20)	
Lesion size, cm^2			**.013**
<3.0	8 (14.5)	1.00	
3.0-5.9	24 (43.6)	2.88 (0.30-27.97)	
6.0-8.9	18 (32.7)	11.00 (1.10-109.67)	
≥9.0	5 (9.1)	28.00 (1.35-580.59)	
Lesion location			.465
Medial femoral condyle	29 (52.7)	1.00	
Lateral femoral condyle	17 (30.9)	2.14 (0.63-7.26)	
Trochlea	9 (16.4)	1.52 (0.33-6.96)	

[a]Boldface indicates statistical significance ($P < .05$).

MSCs isolated from Lewis and Brown Norway rats in the younger (<4 weeks old) and senior (>15 months old) groups, and they reported that the yield of MSCs was significantly higher in the younger group than in the senior group ($P < .02$).

There were significant differences in clinical outcomes at final follow-up among the 3 age groups in this study ($P < .05$) (Table 2). Interestingly, we also found that the age of 60 years could be a useful cutoff value for obtaining encouraging outcomes after MSC implantation (Figure 1, A and B). Furthermore, we found that age was an independent predictor of clinical failure after MSC implantation ($P = .003$) (Table 7). We speculated that these findings may have resulted from a less favorable quality in the cartilage repair of MSCs from patients aged >60 years. Accordingly, we concluded that the patient age should be considered when using recipient MSCs in clinical applications.

Patient obesity is another risk factor for OA.[14] According to a previous report, MSCs from obese patients showed a reduced proliferation rate, greater cell senescence, and reduced differentiation to multiple lineages, including chondrogenesis.[40] In our previous studies,[26,28] we reported that a high BMI (≥27.5 kg/m^2) was a poor prognostic factor when MSC implantation was performed without a scaffold. However, we found that the negative effects in patients with a high BMI can be overcome by using fibrin glue as a scaffold.[26] In the current study, we divided the patients according to their BMI, and we found that there were no significant differences in the clinical outcomes at final follow-up among the groups of BMI (Table 4). Although the exact reason for these findings cannot be explained, we believe that the fibrin glue scaffold may have affected them. Fibrin glue, which has been widely used as a cell delivery matrix for articular cartilage repair,[1,10,13,22] promotes the proliferation and gene expression of MSCs.[19] In an in vitro human study, Kim et al[25] reported that adipose-derived MSCs in fibrin glue sustained functional survival and paracrine function, and they suggested further development of the MSCs with fibrin glue for clinical treatment. Therefore, in the present study, we considered that using fibrin glue as a scaffold may have induced better cell survival, proliferation, differentiation, and matrix synthesis, which led to the symptom improvement in patients with degenerative lesions.

Many studies have reported on the prognostic factors of cartilage regenerative procedures such as arthroscopic microfracture surgery or autologous chondrocyte implantation in chondral defects of knee, and strong correlations between the lesion size and clinical outcomes have been reported by several authors.[2,16,18,39,42] Salzmann et al[42] reported that microfracture surgeries are usually performed to treat lesions <3 cm^2 in size, and Knutsen et al[27] indicated that full-thickness chondral defects <4 cm^2 respond better to microfracture surgery than lesions >4 cm^2. It is well documented that the average size of a lesion treated with autologous chondrocyte implantation is about 5.3 cm^2,[39] and several authors demonstrated better clinical outcomes after autologous chondrocyte implantation compared with microfracture surgery in patients with lesions >4 cm^2.[5,27] However, although several authors have performed microfracture surgery to treat knees with OA,[3,23,32,41] only

a few have reported on the lesion size as a prognostic factor in using microfracture surgery to treat knees with OA. Kaul et al[23] performed microfracture surgery in 5 patients with early OA (Kellgren-Lawrence[24] grade 1-2) and reported that the microfracture surgery did not improve small cartilage lesions (range, 2.6-4.0 cm^2). Sakata et al[41] demonstrated that microfracture surgeries are not suitable for treating cartilage lesions >6 cm^2 in the medial compartmental OA of the knee.

In our previous studies,[26,28] we performed MSC implantation in patients with knee OA, and we found that patients with lesion >5.4 cm^2 and >5.7 cm^2 showed significantly worse outcomes in terms of the IKDC score and Tegner activity score. In the present study, we assessed whether the cartilage lesion size influenced the clinical outcomes of MSC implantation. Accordingly, we found significant differences in the clinical outcomes at final follow-up among the lesion size groups (Table 5). Furthermore, we found that patients with lesions >6.0 cm^2 showed less favorable clinical outcomes after MSC implantation compared with lesions <6.0 cm^2 (Figure 1, C and D). On the basis of our findings, we can suggest a lesion size of 6.0 cm^2 as an upper limit for obtaining encouraging outcomes after MSC implantation. However, there is an inherent point that should be considered. In this study, there was a statistically significant association between age and lesion size (CC = 0.471, $P < .001$) (online Appendix): Older patients were more likely to have larger lesions and therefore worse clinical outcomes. Therefore, further study comparing the outcomes between different age groups with similar lesion sizes is needed to evaluate the independent effect of lesion size on clinical outcomes after MSC implantation.

The current study has some limitations. First, the number of patients was relatively small, and the follow-up period was short. However, given that there are no similar studies published, we believe that these data are important. Furthermore, because this study is ongoing, it can be strengthened in the future, as the number of patients in the matched groups will increase over time. Second, the current study was a retrospective case series that lacked any comparative cohort or control. A comparative study of MSC implantation to other cartilage regenerative procedures is required to identify the exact effects of MSC implantation in cartilage regeneration. In addition, we used the IKDC and Tegner activity scores to evaluate clinical outcomes. Follow-up MRI or second-look arthroscopy with histological evaluation would be helpful for assessment of the quality of repaired cartilage, and further, power analyses of correlations of these findings with clinical outcomes are necessary to identify prognostic factors more precisely. In the current study, the relatively small number of patients may have limited the power to detect other prognostic factors. Third, the quality of MSCs needed to achieve the optimal response in MSC implantation remains unknown. Niemeyer et al[35] analyzed the influence of cell quality on clinical outcomes after autologous chondrocyte implantation in patients with cartilage defects of the knee joint, and they compared its influence quantitatively with patient-specific and defect-specific parameters. A future study that estimates the quality of MSCs that influence the clinical outcomes of MSC implantation is needed to more accurately assess the influential prognostic factors. Furthermore, the number of MSCs used for implantation is important. Jo et al[21] performed intra-articular injection of adipose-derived MSCs for knee OA. The study consisted of 3 dose-escalation cohorts: low dose (1.0×10^7 cells), mid-dose (5.0×10^7), and high dose (1.0×10^8), each with 3 patients. These authors found that the radiological, arthroscopic, and histological outcomes of intra-articular injection of 1.0×10^8 MSCs into the knee OA demonstrated a decrease in the articular cartilage defects by regeneration of hyaline-like articular cartilage. Therefore, further studies are required to determine the optimal number of MSCs by comparing the effects of MSCs on better cartilage regeneration to achieve better clinical outcomes. Fourth, in the current study, fibrin glue was used as a scaffold in MSC implantation. Although the biomechanical properties of an optimized scaffold may provide an environment for promoting the differentiation of stem cells toward the chondrocyte lineage,[7] refinement of the chemical and material properties of scaffolds may improve the biological cues required for infiltration and proliferation of MSCs in scaffolds. Therefore, the development of novel scaffolds that mimic the inherent gradient structure of healthy cartilage may improve cellular activity in tissue engineering-mediated cartilage repair, and comparing the effects of various scaffolds on the clinical outcomes after MSC implantation will strengthen this study. Lastly, in this study, partial weightbearing was initiated at 2 weeks postoperatively, which might be a relatively short duration allowing for adequate cartilage regeneration. Therefore, further study comparing the influence of early and delayed weightbearing on the outcomes after MSC implantation is needed.

CONCLUSION

The current study showed encouraging clinical outcomes after MSC implantation in patients with knee OA. Furthermore, patient age and lesion size were important factors that affected the clinical outcomes. When the patient age was >60 years or the lesion size was >6.0 cm^2, there were less favorable clinical outcomes. Identifying these factors may provide a more accurate screening tool for surgeons to better assess which patients are good candidates for MSC implantation and which will have a better chance at successful clinical outcomes.

REFERENCES

1. Ahmed TA, Giulivi A, Griffith M, Hincke M. Fibrin glues in combination with mesenchymal stem cells to develop a tissue-engineered cartilage substitute. *Tissue Eng Part A*. 2011;17:323-335.
2. Badri A, Burkhardt J. Arthroscopic debridement of unicompartmental arthritis: fact or fiction? *Clin Sports Med*. 2014;33:23-41.
3. Bae DK, Yoon KH, Song SJ. Cartilage healing after microfracture in osteoarthritic knees. *Arthroscopy*. 2006;22:367-374.
4. Barry F, Murphy M. Mesenchymal stem cells in joint disease and repair. *Nat Rev Rheumatol*. 2013;9:584-594.
5. Basad E, Ishaque B, Bachmann G, Sturz H, Steinmeyer J. Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study. *Knee Surg Sports Traumatol Arthrosc*. 2010;18:519-527.

6. Bodle JC, Teeter SD, Hluck BH, Hardin JW, Bernacki SH, Loboa EG. Age-related effects on the potency of human adipose-derived stem cells: creation and evaluation of superlots and implications for musculoskeletal tissue engineering applications. *Tissue Eng Part C Methods*. 2014;20:972-983.
7. Caldwell KL, Wang J. Cell-based articular cartilage repair: the link between development and regeneration. *Osteoarthritis Cartilage*. 2015;23:351-362.
8. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem*. 2006;98:1076-1084.
9. Chang HX, Yang L, Li Z, Chen G, Dai G. Age-related biological characterization of mesenchymal progenitor cells in human articular cartilage. *Orthopedics*. 2011;34:e382-e388.
10. Chang J, Rasamny JJ, Park SS. Injectable tissue-engineered cartilage using a fibrin sealant. *Arch Facial Plast Surg*. 2007;9:161-166.
11. Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. *J Transl Med*. 2014;12:8.
12. Cucchiarini M, Madry H. Use of tissue engineering strategies to repair joint tissues in osteoarthritis: viral gene transfer approaches. *Curr Rheumatol Rep*. 2014;16:449.
13. Dare EV, Griffith M, Poitras P, et al. Fibrin sealants from fresh or fresh/frozen plasma as scaffolds for in vitro articular cartilage regeneration. *Tissue Eng Part A*. 2009;15:2285-2297.
14. Diekman BO, Guilak F. Stem cell-based therapies for osteoarthritis: challenges and opportunities. *Curr Opin Rheumatol*. 2013;25:119-126.
15. Dos-Anjos Vilaboa S, Navarro-Palou M, Llull R. Age influence on stromal vascular fraction cell yield obtained from human lipoaspirates. *Cytotherapy*. 2014;16:1092-1097.
16. Ebert JR, Smith A, Edwards PK, Hambly K, Wood DJ, Ackland TR. Factors predictive of outcome 5 years after matrix-induced autologous chondrocyte implantation in the tibiofemoral joint. *Am J Sports Med*. 2013;41:1245-1254.
17. Goldring MB. Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. *Ther Adv Musculoskelet Dis*. 2012;4:269-285.
18. Harris JD, Siston RA, Pan X, Flanigan DC. Autologous chondrocyte implantation: a systematic review. *J Bone Joint Surg Am*. 2010; 92:2220-2233.
19. Ho W, Tawil B, Dunn JC, Wu BM. The behavior of human mesenchymal stem cells in 3D fibrin clots: dependence on fibrinogen concentration and clot structure. *Tissue Eng*. 2006;12:1587-1595.
20. Irrgang JJ, Anderson AF, Boland AL, et al. Development and validation of the International Knee Documentation Committee subjective knee form. *Am J Sports Med*. 2001;29:600-613.
21. Jo CH, Lee YG, Shin WH, et al. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *Stem Cells*. 2014;32:1254-1266.
22. Jung SN, Rhie JW, Kwon H, et al. In vivo cartilage formation using chondrogenic-differentiated human adipose-derived mesenchymal stem cells mixed with fibrin glue. *J Craniofac Surg*. 2010;21:468-472.
23. Kaul G, Cucchiarini M, Remberger K, Kohn D, Madry H. Failed cartilage repair for early osteoarthritis defects: a biochemical, histological and immunohistochemical analysis of the repair tissue after treatment with marrow-stimulation techniques. *Knee Surg Sports Traumatol Arthrosc*. 2012;20:2315-2324.
24. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis*. 1957;16:494-502.
25. Kim I, Lee SK, Yoon JI, Kim da E, Kim M, Ha H. Fibrin glue improves the therapeutic effect of MSCs by sustaining survival and paracrine function. *Tissue Eng Part A*. 2013;19:2373-2381.
26. Kim YS, Choi YJ, Suh DS, et al. Mesenchymal stem cell implantation in osteoarthritic knees: is fibrin glue effective as a scaffold? *Am J Sports Med*. 2015;42(1):176-185.
27. Knutsen G, Engebretsen L, Ludvigsen TC, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. *J Bone Joint Surg Am*. 2004;86:455-464.
28. Koh YG, Choi YJ, Kwon OR, Kim YS. Second-look arthroscopic evaluation of cartilage lesions after mesenchymal stem cell implantation in osteoarthritic knees. *Am J Sports Med*. 2014;42:1628-1637.
29. Koh YG, Choi YJ, Kwon SK, Kim YS, Yeo JE. Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(5):1308-1316.
30. Koh YG, Jo SB, Kwon OR, et al. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. *Arthroscopy*. 2013;29: 748-755.
31. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J Bone Joint Surg Am*. 2007;89:780-785.
32. Lee GW, Son JH, Kim JD, Jung GH. Is platelet-rich plasma able to enhance the results of arthroscopic microfracture in early osteoarthritis and cartilage lesion over 40 years of age? *Eur J Orthop Surg Traumatol*. 2013;23:581-587.
33. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum*. 2012;64: 1697-1707.
34. Luyten FP, Denti M, Filardo G, Kon E, Engebretsen L. Definition and classification of early osteoarthritis of the knee. *Knee Surg Sports Traumatol Arthrosc*. 2012;20:401-406.
35. Niemeyer P, Pestka JM, Salzmann GM, Sudkamp NP, Schmal H. Influence of cell quality on clinical outcome after autologous chondrocyte implantation. *Am J Sports Med*. 2012;40:556-561.
36. Noth U, Steinert AF, Tuan RS. Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. *Nat Clin Pract Rheumatol*. 2008;4:371-380.
37. Oreffo RO, Cooper C, Mason C, Clements M. Mesenchymal stem cells: lineage, plasticity, and skeletal therapeutic potential. *Stem Cell Rev*. 2005;1:169-178.
38. Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: two-year follow-up results. *Transplantation*. 2014;97:e66-e68.
39. Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: a long-term follow-up. *Am J Sports Med*. 2010;38:1117-1124.
40. Roldan M, Macias-Gonzalez M, Garcia R, Tinahones FJ, Martin M. Obesity short-circuits stemness gene network in human adipose multipotent stem cells. *FASEB J*. 2011;25:4111-4126.
41. Sakata K, Furumatsu T, Abe N, Miyazawa S, Sakoma Y, Ozaki T. Histological analysis of failed cartilage repair after marrow stimulation for the treatment of large cartilage defect in medial compartmental osteoarthritis of the knee. *Acta Med Okayama*. 2013;67:65-74.
42. Salzmann GM, Niemeyer P, Steinwachs M, Kreuz PC, Sudkamp NP, Mayr HO. Cartilage repair approach and treatment characteristics across the knee joint: a European survey. *Arch Orthop Trauma Surg*. 2011;131:283-291.
43. Tegner Y, Lysholm J. Rating systems in the evaluation of knee ligament injuries. *Clin Orthop Relat Res*. 1985;198:43-49.
44. Wu LW, Wang YL, Christensen JM, et al. Donor age negatively affects the immunoregulatory properties of both adipose and bone marrow derived mesenchymal stem cells. *Transpl Immunol*. 2014;30:122-127.