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Efficacy and safety of autologous adipose tissue-derived stem cell therapy for children with refractory Crohn's complex fistula: a Phase IV clinical study

Yu Jeong Cho[1], Hyunhee Kwon[1], Yong Jae Kwon[1], Kyung Mo Kim[2], Seak Hee Oh[2], Dae Yeon Kim[1]

[1]Department of Pediatric Surgery, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea
[2]Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea

Purpose: Autologous adipose tissue-derived stem cells (ASCs) have been proposed for patients with refractory Crohn disease, but research is lacking in pediatric patients. This Phase IV study evaluated the efficacy and safety of ASCs in children with refractory Crohn's fistulae.
Methods: Patients with a refractory Crohn's fistula who did not have conventional therapy for more than 3 months or with a recurrent complex Crohn's fistula were included. All patients were at least 14 years old. Patients with infection, poor condition, or active Crohn disease with a disease activity index of 450 and above were excluded. Five patients were treated with ASCs from 2014 to 2015 in Asan Medical Center. ASC administration was adjusted according to fistula size (1 mL per cm^2). We evaluated the efficacy and safety 8 weeks after injection and followed patients for 6 months.
Results: Fistulae were healed in 4 patients by 8 weeks after ASC injection. Of these 4 patients, 1 had complete fistula closure and sustainability after 6 months. The other 3 with healing effects had less than 50% fistula closure by 6 months. None of these 4 patients have persistent fistulae. One patient had no healing effect, and seton ligation was performed 8 months after ASC injection. There were no adverse effects related to ASC administration.
Conclusion: ASC therapy is a simple and well-tolerated therapeutic option for children with refractory Crohn's complex fistulae. Complete closure was well-sustained. However, more data from a larger number of patients are needed.
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Key Words: Autologous stem cells, Mesenchymal stem cells, Pediatric Crohn's disease

INTRODUCTION

Crohn disease (CD) is a complex disorder of multifactorial etiology characterized by chronic recurrent inflammation of the gastrointestinal tract. The exact reasons for the disease are not yet known, and it is assumed that genetic factors, environmental factors, and immune factors due to microbial infection are involved [1]. Compared to the incidence of CD in Europe, Korea, and other Asian countries are considered relatively low-incidence areas (4.0–7.0 *vs.* 1.34 per 100,000 persons) [2,3]. Comprehensive population-based studies are lacking in adults, but about 25% of patients with inflammatory bowel disease are diagnosed before 18 years of age [4]. A recent study in Korea and other countries showed a rapid increase in CD incidence in children as well as adults [2].

Perianal perforating CD is reported in approximately 8%–13% of children with CD at the time of diagnosis [5]. Fistulae frequently invade the anal sphincter muscle complex and can

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Corresponding Author: Dae Yeon Kim
Department of Pediatric Surgery, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-6841, **Fax:** +82-2-474-6701
E-mail: kimdy@amc.seoul.kr
ORCID: https://orcid.org/0000-0001-6130-1796

involve other pelvic structures, such as the urethra, vagina, labia, scrotum, and bladder. Furthermore, these symptoms can lead to serious complications, such as abscesses and fecal incontinence [6,7]. Medical treatment for CD fistulae initially focused on surgical intervention, along with the treatment of symptoms using antibiotics and immunosuppressants. Medical and surgical treatments are often only partially effective, and recurrence of perianal fistulae is common [8]. CD fistulae are considered to be more frequent and aggressive in patients with pediatric-onset CD [9]. CD fistulae based on biological agents such as infliximab generally do not close completely. Despite the closure of external draining orifices, residual inflammation in the fistula tract after infliximab therapy was suggested to be the primary reason for recurrence [10]. To achieve a beneficial additive effect, combination treatment with infliximab and surgical intervention is recommended for the care of CD fistulae.

The ideal therapeutic goal in treating perianal CD fistulae is complete and sustained closure of the fistulae without recurrence, as well as the preservation of the anal sphincter. The surgical interventions include long-term seton placement for drainage, fibrin glue injection, and a fistula plug, but complete healing rates are <50% [11]. Improved healing rates can be achieved with rectal advancement flaps and ligation of the internal fistula tract, but they have an increased risk of incontinence and cannot be performed in cases of accompanying proctitis or multiple fistula tracts, which are characteristic of perianal CD fistulae [11,12].

Autologous adipose tissue-derived stem cells (ASCs) have been used in treating perianal CD. These stem cells have anti-inflammatory, immunomodulatory, and fibroblast-like healing effects [13-15]. ASCs are thought to be a safe and effective therapy for the treatment of CD fistulae. ASCs do not cause fecal incontinence after injection into the lesion site. Overall, healing was observed in 33%–89% of cases, with continued remission after 6 months to a year in a clinical study of patients with complex fistulae [16].

Prior to this Phase IV clinical study, an adult clinical study with ASCs manufactured by Anterogen Corp., Ltd. (Seoul, Korea) demonstrated the therapeutic potential and safety for the treatment of CD fistulae [17]. This study was carried out to evaluate the efficacy and safety of ASCs in children with refractory CD fistulae, which are characteristic of pediatric-onset CD.

METHODS

Patients

Eligible patients were children over 14 years old and with a body mass index of >17 kg/m^2 who were diagnosed with CD and with the consent to participate in the study from a legal representative. Patients with refractory CD fistulae who were not treated with conventional therapy for more than 3 months or with recurrent complex CD fistula were included in the clinical trial. Complex fistula was defined as high trans-sphincteric with involvement of more than 30% of the anal sphincter, suprasphincteric extrasphincteric, and multiple openings [18]. Patients were not eligible for inclusion if they fulfilled at least one of the following conditions: a medical or family history of variant Creutzfeldt-Jakob disease; autoimmune diseases or inflammatory bowel disease other than CD; activated severe CD (CD activity index > 450); infectious diseases including HBV, HCV, and HIV infection; active tuberculosis (including anal tuberculosis); signs of septicemia; allergies or hypersensitivity to bovine-derived materials; sensitivity to fibrin glue; or surgery for malignancy (except *in situ* carcinoma) during the past 5 years. Patients were also excluded if tissues obtained by liposuction were inadequate for the preparation of scheduled doses.

Study design

Following the approval from the Institutional Review Board of Asan Medical Center (No. 2014-0273), this Phase IV open-label study was conducted in a single center from January 2014 to December 2015 in South Korea. All patients provided written informed consent before initiating the trial. Eligible patients underwent liposuction to acquire fat tissue.

Fat tissue (10–40 mL) was digested in phosphate-buffered saline (HyClone Lab, Logan, UT, USA) containing 1% bovine serum albumin and 0.025% collagenase for 80 minutes at 37°C with intermittent shaking. Isolated stromal vascular fraction was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1 ng/mL basic fibroblast growth factor to get the appropriate number of ASCs for injection. ASCs at passages 3–4 were harvested by trypsin treatment, suspended in DMEM, and packaged in single-use vials containing 3×10^7 cells/mL (Anterogen Corp., Ltd.) [17]. All manufacturing procedures were carried out according to the Good Manufacturing Practices authorized by the Korean Ministry of Food and Drug Safety. Manufactured ASCs expressed the stromal cell-associated markers CD (cluster of differentiation) 10, CD13, CD29, CD44, and CD90. These cells were negative for expressing the hematopoietic stem cell-associated markers CD34 and CD45 and the bone marrow-derived stem cell-associated marker STRO-1 [19]. ASCs confirmed genomic stability through karyotyping, and no evidence of tumor formation was observed in a tumorigenicity study. When analyzed for lot release testing, the minimum criteria were more than 80% for cell viability and less than 1% of CD45-positive cells for purity. Furthermore, ASCs were confirmed for safety against contamination.

The required amounts of ASCs were determined by

measuring the length and diameter of the fistula via examination under anesthesia and MRI. If there were multiple fistulae, a target fistula was determined for evaluation.

The surgical procedure is as follows. We thoroughly removed abscesses and granulated tissues in the fistula before injection with ASCs under anesthesia and then sutured the internal opening with vicryl 4-0. The ASCs were injected using a 24-gage needle in an even dose per surface area around the internal opening and fistula tract and were then blocked by filling the tract with a mixture of ASCs and fibrin glue (GreenPlast, Green Cross, Seoul, Korea). If the shape of the fistula was irregular or if the injection was difficult, it was filled with the mixture. When mixing, fibrin glue was 30% or less of the total mixing volume. The dose of ASCs was determined based on the fistula surface area. When the diameter of the fistula was 1 cm or less, 1 mL of ASCs (3×10^7 cells) was injected per centimeter length and 2 mL of ASCs (6×10^7 cells) were administered when the diameter of the fistula was greater than 1 cm and less than or equal to 2 cm.

After the injection, the administration of immunosuppressants and antibiotics for medical treatment of CD was allowed, but immunosuppressants were not approved for fistula management. In addition, the target fistula did not receive any other procedures or operations other than ASC injection.

Follow-up and evaluation

To assess fistula closure, clinicians imaged the lesion site at each outpatient clinic visit 4 weeks, 8 weeks, and 6 months after ASC injection and compared with images taken on day 1 (before ASC injection) (Fig. 1). In addition to imaging, we prospectively recorded the perianal disease activity index score (PDAI) (no disease, 0; severe disease, 20) to score physical examination findings of perianal disease and evaluate quality of life (QOL), and evaluated the effect after ASC injection through MRI and CT at, before, and 6 months after ASC injection. The PDAI has been shown to precisely assess the degree of impairment and detect important clinical changes, and correlates well with a response to treatment [20]; thus, it is an indicator of QOL. The primary efficacy endpoint of this study was the complete closure of fistulae injected with ASCs, and the secondary efficacy endpoints were a decrease in drainage of more than 50% and clinician and patient satisfaction with ASC efficacy according to a 5-point grading scale. The continuity of the efficacy was assessed at 6 months. The safety evaluation included analysis of systemic tolerance, adverse effects, serious adverse events, and laboratory toxicity after ASC injection.

RESULTS

Five patients were enrolled and injected with ASCs in the study period. Patient characteristics are shown in Table 1. Three patients were male and 2 were female. The median age was 16 years (range, 15–19 years). The median duration of CD was 14 months (range, 7–101 months). The median pediatric CD activity index (PCDAI) was 17 (range, 10–30) and most patients had mild CD activity. The median period of treatment for anal fistulae was 18 months (range, 10–84 months). All fistulae were transsphincteric. The median length of the target fistula was 5 cm (range, 4–15 cm) and the median diameter was 1 cm (range, 0.8–1.5 cm). The median ASC injection volume was 10 mL (range, 8–15 mL), and the median number of ASCs on injection was 30.0×10^7.

The efficacy outcome of ASCs was summarized based on the postinjection period (Table 2). One patient had complete fistula closure at 8 weeks after ASC injection. There were 3 patients with less than 50% fistula closure with a decrease in drainage,

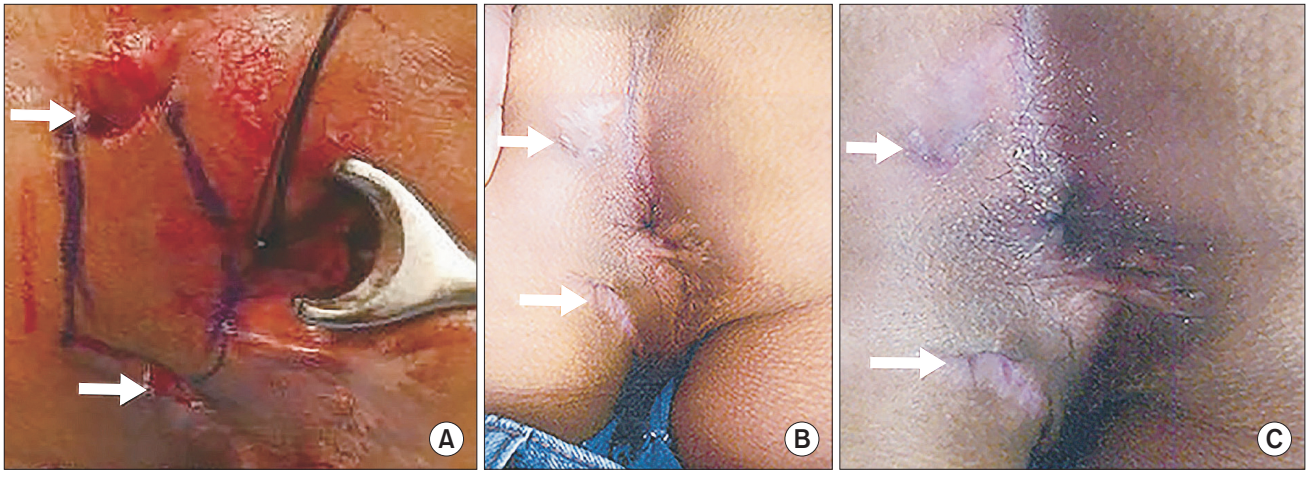

Fig. 1. Patient A. Treatment with autologous adipose tissue-derived stem cells. (A) Before injection. (B) Eight weeks after injection. (C) Six months after injection, showing healed fistula with complete epithelialization of the external opening. The white arrows show the injection site.

Table 1. Demographic and baseline characteristics

Parameter	Patient No.				
	1	2	3	4	5
Sex	Male	Male	Male	Female	Female
Age (yr)	15	16	15	17	19
No. of openings					
Internal	1	1	1	2	2
External	2	1	1	2	3
Type of fistula	Trans	Trans	Trans	Trans	Trans
Diameter of target fistula (cm)	1	0.8	1.5	1	4
Length of target fistula (cm)	4	11	4	15	5
Duration of Crohn disease (mo)	10	7	16	14	101
Duration of fistula (mo)	10	8	16	18	84
History of operation for fistula	1	2	3	5	2
Concomitant medication					
5-ASA	√	√	√	√	
AZA				√	
Anti-TNF-α			√	√	√
PDAI	12	8	12	8	9
PCDAI	10	10	17	30	22.5

5-ASA, 5-aminosalicylate; AZA, azathioprine; PDAI, perianal disease activity index; PCDAI, pediatric Crohn disease activity index; Trans, transsphincteric.

Table 2. Efficacy outcomes of patients at 8 weeks and 6 months

Variable	Patient No.				
	1	2	3	4	5
Closure of fistula (%)					
8 wk	Complete	<50	≥50	<50	<50
6 mo	Complete	≥50	≥50	≥50	<50
PDAI					
8 wk	0	6	2	1	6
6 mo	0	5	7	2	5

PDAI, perianal disease activity index.

and the other one had more than 50% marked decrease in drainage and closure at 8 weeks after ASC injection. The median PDAI was 9.0 (range, 8–12) before injection, but after 8 weeks of injection, it was 2.0 (range, 0–6), confirming that the QOL in all patients had risen. Fistula healing was sustained and improved in 4 patients at 6 months after the injection. Only 1 patient (patient 5) still showed a fistula closure with a mild decrease of <50% at 6 months. The PDAI of this patient was reduced to 5 compared to 9 before ASC injection and the patient showed improved QOL. All patients showed improvement in radiologic evaluation in the target fistula 6 months after ASC administration (Fig. 2).

No ASC-related adverse events were observed. All patients tolerated the treatment well without safety concerns, and the healing effect was sustained without recurrence at 6 months after the injection.

DISCUSSION

Recently, a large body of evidence demonstrated the efficacy of ASC treatments in adult patients with CD and perianal fistulae (Table 3) [15-17,21-23]. Such studies reported that ASCs could be safely used at various concentrations and were tolerable without serious complications. We considered that treatment with ASCs could be an important therapeutic alternative in children, given that fistulae are more common and aggressive in pediatric-onset CD [9]. The primary goal of this study was to verify the efficacy and safety of ASCs in the treatment of CD fistulae in children.

The exact mechanism of perianal fistula healing by ASCs remains unknown. It likely results from a combination of the qualities of ASCs. In the present study, ASCs in CD patients are inhibited in their adipogenic differentiation potential and have anti-inflammatory and immune-modulatory effects [14,15]. Stem cell transplantation is considered a novel treatment for long-term refractory CD [24]. There is accumulating evidence that mesenchymal stem cell (MSC)-mediated immunosuppression may be initiated by the release of proinflammatory cytokines, such as IFN-γ, TNF-a, and activated immune cells [25]. In CD fistulae, the local injection of stem cells is considered to be beneficial with sustained efficacy based on the following findings. The differentiation properties of ASCs are extremely reduced during the expansion process

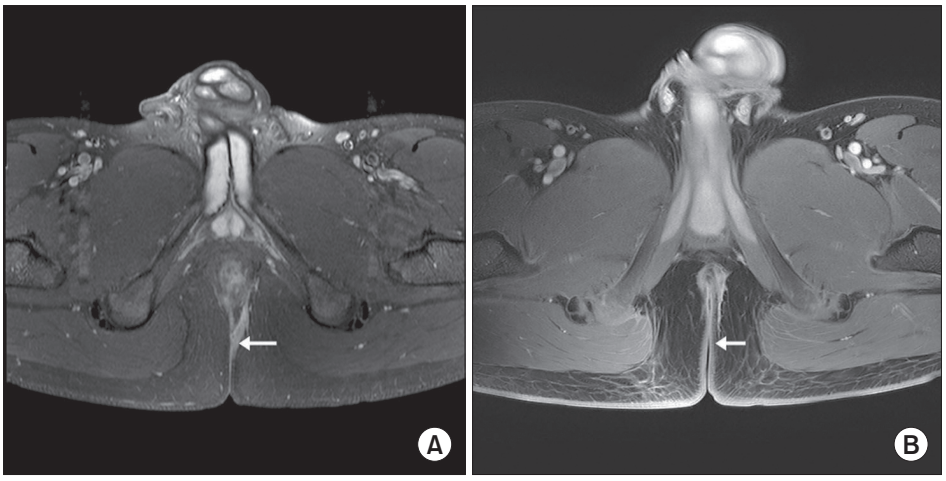

Fig. 2. MRI shows a markedly obliterated fistula tract. (A) Before injection. (B) Six months after injection. The white arrows show the fistula tract.

Table 3. Overview of adipose tissue-derived stem cell studies for the treatment of perianal fistulae in Crohn disease

Study	Year	Study design	Results
Garcia-Olmo et al. [21]	2009	n = 14 (Phase IIb study) Autologous adipose-derived stem cell + fibrin glue *vs*. fibrin alone	Complete closure in 71% *vs*. 14% at 8 weeks
de la Portilla et al. [15]	2013	n = 24 (Phase I/II study) Allogenic adipose-derived stem cell	Complete closure in 56.3% of patients at 24 weeks and improvement in the patients' condition No related severe adverse events
Park et al. [23]	2016	n = 6 (prospective pilot study) Allogenic adipose-derived stem cell + fibrin glue	Complete closure in 16.7% of patients at 8 weeks and 8 months No related adverse events
Turse et al. [16]	2018	Systematic review	Stem cell therapy is safe and effective for patients with perianal disease

while the immunomodulatory and anti-inflammatory effects appear to be intrinsic and unaltered by the expansion. Given that CD involves immune dysregulation associated with chronic and recurrent inflammation with increasing levels of pro-inflammatory cytokines, ASCs may induce an optimal balance in the immune system, and a rebalanced immune system may lead to a sustained response. Ciccocioppo et al. [26] also reported that regulatory T cells increased by MSC treatment remained stable until the 1-year follow-up.

The therapeutic effect of ASCs on complex fistulae in an adult Phase I/II study using a mixture of ASCs and fibrin glue showed a higher therapeutic effect compared to other ASC studies (79% *vs*. 50%–75%), which was because of the use of ASCs and fibrin adhesives in the selected patient populations [17]. It is expected that fibrin glue would provide an additional effect on fistula healing by increasing cell transplant survival rate and having a lasting effect on the submucosa of the fistula tract. The effect of fibrin glue may be due to the following findings. Fibrin activates fibroblasts and endothelial cells. Collagen synthesis initiated by fibroblasts and angiogenesis by endothelial cells can aid healing; however, using fibrin glue alone reduced the therapeutic effect on fistulae [27]. In 2009, Garcia-Olmo et al. [21] reported that MSCs with fibrin glue for complex perianal fistulae had significant efficacy versus fibrin glue alone (71% *vs*. 16%, $P < 0.001$).

Given that administration of biologic therapies, such as infliximab, are for long-term use and have more reported adverse events such as serum sickness-like reaction, opportunistic infections, sepsis with just a 50% resolution, and common recurrence of fistulae [28]. ASCs could be a promising treatment for CD fistulae. Another treatment for patients with high trans-sphincteric or complex fistulae is an endorectal mucosal advancement flap, which has poor healing capabilities and a high rate of fistula recurrence with postoperative anal incontinence caused by sphincter injury [29]. In seton treatment of highly complex fistulae, long-term seton drainage preserves sphincter function, but recurrence is common if the seton is removed.

Source tissue for ASCs is simple to obtain through liposuction (compared to bone marrow stem cells), and it can be collected in large amounts and can be expanded *in vitro* [30]. However, patients with long-standing CD lose weight due to malnutrition,

which may make it difficult to obtain sufficient fat tissue. If the appropriate patient is selected based on general condition and physical examination, allogenic ASCs have the advantage of being obtainable in sufficient numbers.

One of the limitations of this study was that a small number of patients was used. This was because patients had to be carefully selected for ASC treatment. Also, it was not easy to obtain patients' or their parents' consent for this emerging therapy. For the same reason, we did not identify any randomized controlled studies and it is unlikely that any will be initiated in the near future, although larger-scale retrospective studies on pediatric CD fistulae are more feasible options that would provide valuable information considering the promising results of current studies.

In conclusion, ASC treatment might be an effective and well-tolerated therapeutic option in pediatric patients with refractory CD complex fistulae who did not respond to conventional treatments. Considering that perianal disease is more common and severe in patients with pediatric CD, it would be better to consider ASCs as a therapeutic option with proper indications. However, larger studies are needed to evaluate its effects, which should determine standardized protocols in children.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

ORCID iD

Yu Jeong Cho: https://orcid.org/0000-0001-6823-2746
Hyunhee Kwon: https://orcid.org/0000-0001-6647-9155
Yong Jae Kwon: https://orcid.org/0000-0001-9490-1229
Kyung Mo Kim: https://orcid.org/0000-0001-7896-6751
Seak Hee Oh: https://orcid.org/0000-0002-9672-8877
Dae Yeon Kim: https://orcid.org/0000-0001-6130-1796

Author Contribution

Conceptualization: All authors
Formal analysis: YJC
Investigation: YJC, DYK
Methodology: KMK, SHO, DYK
Project Administration: KMK, DYK
Writing – Original Draft: YJC, DYK
Writing – Review & Editing: All authors

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