Short-term results of adipose-derived stem cell therapy for the treatment of complex perianal fistula

A single center experience

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BACKGROUND: *In this study, we aimed to investigate the early results of the safety and efficacy of adipose-derived stem cells (ADSCs) injection along with the repair of the internal orifice in the recovery of complex perianal fistula.*
METHODS: *The study included patients who underwent autologous adipose tissue-derived stemcell injection for complex perianal fistula between December2017 and January2018. The FDA-approved Lipogems® system was used to prepare autologous micro-fragmented adipose stem cells. Demographic characteristics, history of inflammatory bowel disease, fistula type and length of fistula tract determined by endoanal ultrasound, mean operation duration, postoperative complications,and fistula healing of the patients were evaluated. Fistula healing was evaluated on the 30th ,90th days and 9th months.*
RESULTS: *A total of 10 patients, with male predominance, were included in our study. The mean age was 47±13.1 and mean BMI was 28.3±4.79. None of the patients had inflammatory bowel disease Based on endoanal ultrasound findings, fistula type was transsphincteric, extrasphincteric and suprasphincteric for 7, 2 and 1 patients, respectively. Calculated length of fistula tract based on ultrasound was 4.45±1.69(2.5-6.4). Mean duration of operation was 45±7 minutes. None of the patients had any treatment related toxicity, however, two patients experienced bruising at the liposuction site. Healing rate in the follow-up of 30-day, 90-day and 9-month was 70%, 80% and 70%, respectively.*
CONCLUSIONS: *In addition to surgical treatment in the form of curettage and closure of the fistula tract, autologous micro-fragmented adipose tissue injection is a safe, feasible, and reproducible procedure that can be performed based on the early results in complex anal fistula healing.*

KEY WORDS: Lipogems®, Mesenchymal stem cell, Regenerative medicine, Adipose tissue-derived mesenchymal stem cell

Introduction

Anal fistulas, defined as abnormal channels between the rectum and surrounding structures, are still a major problem in daily surgical practice. Perianal fistula is one of the most common anorectal diseases with an annual incidence of 8-10 cases per 100,000 people [1].
Mesenchymal stem cells were first defined by Friedenstein in 1974, however it took years for this term to gain widespread acceptance [2]. The main harvesting source of these cells are known to be medulla spinalis and fat tissue [2,3]. Liposuctioned fat is available in large quantities and can be harvested with minimal inconvenience to the patient [4,5]. In routine classification, stem cells are divided into 2 main groups as adult and embryonic stem cells. Adult stem cells are used more frequently in treatments because of their easy access from different tissues and a more limited controlled differentiation potential. Although the mechanisms are still poorly understood, tissues have been shown to promote angiogenesis and prevent fibrosis in addition to their ability to regenerate [6].

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The use of stem cells in the treatment of complex fistulas is a promising research area. Over the past thirty years we have made great progress in regenerative medicine and stem cell biology. After the discovery of lipoaspirate cells as an ideal stem cell source differentiating into myogenic cells, adipose-derived stem cells (ASDCs) started to be used in the treatment of anal fistula [7].
The first published case of stem cell application was with a 32-year-old patient with Crohn's disease, with a recurring rectovaginal fistula [8]. The aim of this study is to present the early period results of adipose-derived stem cells (ASDCs) application with internal orifice repair in complex anal fistula healing.

Material and Method

Patients who underwent autologous adipose tissue-derived stem cell injection for complex perianal fistula between December 2017 and January 2018 in the Proctology Unit of the General Surgery Department of Çukurova University Faculty of Medicine were included in the study.
A common database was created by examining patient files and hospital information system records. Using this database, patient information was evaluated retrospectively. Patients whose records could not be reached were excluded from the study. The choice of complex fistula was according to the definition of the American Gastroenterological Association (AGA). Parks classification was used for fistula classification [9,10].
All patients underwent endoanal USG for preoperative fistula mapping. Endoanal USG device Flex focus 400 (Bruel and Kajarer, Medical USG scanner, Herlev, Denmark) 360-degree probe with a frequency range of 16-6 MHz was used.
Demographic characteristics, comorbidities, history of inflammatory bowel disease, history of fistula surgery, fistula type and length of fistula tract determined by endoanal ultrasound, intra-operative evidence of fistula internal and external orifice localization in lithotomy position, mean operation duration, [0-10 visual analog scale (VAS)], postoperative complications, postoperative hospitalisation duration, readmission to the hospital after discharge evaluated.
Fistula healing was defined as the closure of the internal and external openings without any discharge. Follow-up visits were made on the 7th, 30th and 90th days and 9 months postoperatively. Fistula healing was evaluated at 30th , 90th days and 9th month.
Before the operation, the patients were informed about the operation and written consent was obtained. The study was conducted according to the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines.

Surgical Technique

Operations were performed in the lithotomy position under spinal anesthesia. The surgical technique was based on pilot studies and phase 2 studies described in the literature [11,12].
The external opening and fistula tract were identified and cleaned using tract curettages, (Fig. 1). and the internal opening was closed by sutures using 2-0 vicryl before ASC injection. Afterwards, adipose tissue was harvested. The lower abdomen was chosen as the donor site for adipose tissue harvesting. Before harvesting the fat, the subcutaneous fat of the anterior abdominal wall was injected with 360 ml (180 ml in each side right and left) of modified Klein solution (500 ml of saline solution, 20 ml lidocaine 20 mg/ml, 1 ml adrenaline 1 mg/ml) using a disposable cannula (Fig. 2).

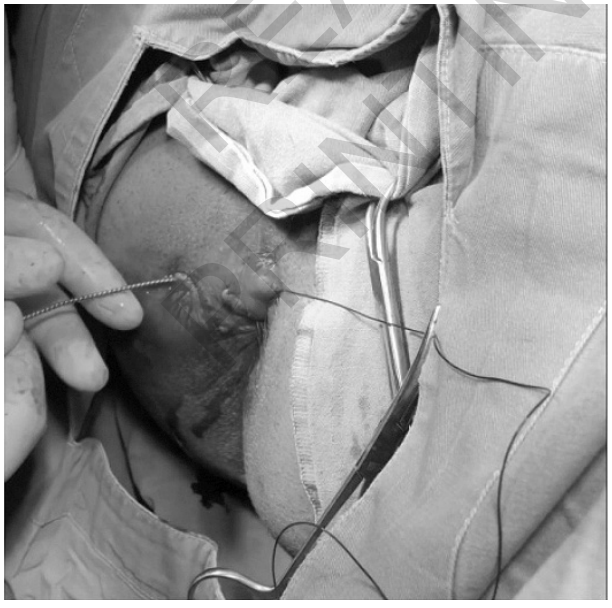

Fig. 1: Debridement of fistula tract.

Fig. 2: Infiltration of the subcutaneous tissue with modified Klein solution

Fig. 3: Liposuction is performed through a cannula (13G) connected to a Vaclock®

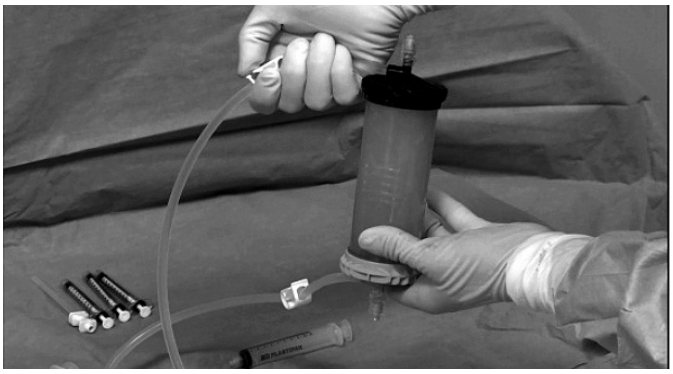

Fig. 5: Injection of the final Lipogems® product through 10-ml syringes around the fistula tract.

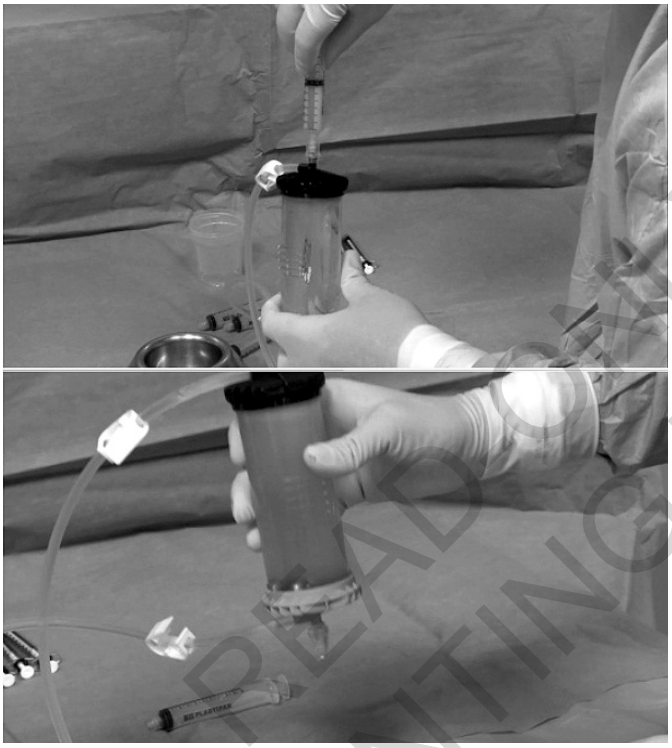

Fig. 4: The syringe with the adipose tissue aspirate is connected to the blue filter pushing it inside the processing cylinder. Both hoses are opened to allow a continuous flow of saline solution and the elimination of oily and bloody residual. The cylinder is shaken until the saline solution inside it becomes clear and then both hoses are closed.

The adipose tissue was harvested using a 13G blunt cannula connected to a Vaclock ® 20-ml syringe (Fig. 3). The aspirated tissue was then collected in 10-ml syringes and positioned for decant. The harvested fat was then processed in the Lipogems® processing kit, a disposable device that gradually reduces the size of the adipose tissue clusters while eliminating oily substances and blood residues with pro-inflammatory properties. The entire process was performed in complete immersion in saline solution minimizing any traumatic action on cell products (Fig. 4).

The resulting microfragmented adipose tissue was collected in a 10-ml syringe and positioned for decant the excess of saline solution. The product was injected evenly using a 10-mL syringe equipped with a 24-gauge needle into the submucosal layer surrounding the internal opening and inside of the fistula tract wall and 4 or more times of dividing injections at all quadrants of fistula wall could distribute the ADSCs evenly around the tract. Injection was very superficial, no deeper than 2 mm (Fig. 5).

Although the doses of ASCs, the cell counts per mL, were randomly determined, the amount of product injected may vary depending on the total amount harvested, but it should not be less than 6 ml, at least 2 ml per cm of fistula tract. We injected an average of 10ml of product.

Statistical Analysis

IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA) package program was used for the statistical analysis of the data. Categorical measurements were summarized in numbers and percentages, and continuous measurements were summarized as mean and standard deviation (median and minimum-maximum where necessary).

Results

A total of 10 patients were included in our study. 2 (20%) were female. Mean age was 47±13.1 (37-69). Mean BMI was 28.3±4.79 (22.5-37.3). As comorbidities, 2 (20%) patients had Type 2 diabetes mellitus, 1

Table I - *Demographic and baseline characteristics*

Parameters	Total (N = 10)
Gender (M-F)	(8-2)
Age Mean± SD (Min-Max)	47±13,1(37-69)
Body mass index Mean± SD (Min-Max)	28,3±4,79(22,5-37,3)
Comorbid disease, n (%)	
Type 2 Diabetes Mellitus (DM)	2 (20)
Chronic Obstructive Pulmonary Disease (COAH)	2 (20)
Previous fistula surgery, n (%)	
Seton	2 (20)
LIFT (Ligation of the Intersphinteric Tract)	2 (20)
Fistulotomy	1 (10)
Length of fistula (cm) Mean±SD (Min-Max)	4,45±1,69(2,5-6,4)
Fistula type, n (%)	
Trans Sphincteric	7(70)
Extrasphincteric	2(20)
Suprasphincteric	1(10)

Table II - *Operation details Complications and Readmission*

Parameters	Total (N = 10)
Fistula External Orifice, n (%)	
12 o'clock	2 (20)
9 o'clock	2 (20)
7 o'clock	1 (10)
6 o'clock	2(20)
3 o'clock	2 (20)
1 o'clock	1(10)
Fistula Internal Orifice, n (%)	
6 o'clock	6 (60)
4 o'clock	2 (20)
1 o'clock	2 (20)
Operation Time Mean±SD (Min–Max)	45±7 (40-80)
Visual Analogue Scales (Vas) Mean±SD (Min–Max)	2±1,2 (0-4)
Postoperative Hospital Stay Time (Day) Mean±SD (Min–Max)	2±0,66 (1-3)
Postoperative Complications, n (%)	
Ecchymosis	2 (20)
30-Day Readmission	2 (20)
Perianal Abscess	2 (20)

Table III - *Efficacy results*

Parameters Results of healing	Total (N=10) n (%)
30-day	7 (70)
90-day	8 (80)
9-month	7 (70)

(10%) patient had chronic obstructive pulmonary disease. None of the patients had inflammatory bowel disease. 2 (20%) patients underwent seton application, 2 (20%) patients underwent *LIFT* (Ligation of the Intersphincteric Tract) and 1 (10%) underwent fistulotomy. According to endoanal ultrasound findings, fistula types were 7 (70%) transsphincteric, 2 (20%) extrasphincteric, and 1 (10%) suprasphincteric. The mean measured length of fistula tract was 4.45± 1.69 (2.5-6.4). It is shown in Table I.

Determined intraoperatively in the lithotomy position, the fistula external orifices were located at 12 o'clock (n:2, 20%), 9 o'clock (n:2, 20%), 7 o'clock (n:1, 10%), 6 o'clock (n:2, 20%), 3 o'clock (n:2, 20%), and 1 o'clock (n:1, 10%). Internal orifices were located at 6 o'clock (n:6, 60%), 4 o'clock (n:2, 20%) and 1 o'clock (n:2, 20%). Operation duration was 45±7 minutes (40-80). Visual analogue scales (VAS) 2±1,2 (0-4). Postoperative duration of hospital stay was 2±0,66 days (1-3). 2 cases had ecchymosis on the liposuction site as postoperative complications. No procedure-related toxicity was observed. Two patients were admitted for perianal abscess within 30 days of discharge. It is shown in Table 2.

Healing rate in 30-day follow up was 7 (70%) ,it was 8 (80%) in 90-day follow up and it was7 (70%) in 9 th month follow up. It is shown in Table III.

Discussion

Although the perianal fistula classification varies, the most simple and practical classification is Parks. It takes internal and external sphincters as reference points. 1-Intersphincteric: Moves down the intersphincteric plane and opens into the skin around the anal verge. 2-Transsphincteric: Passes the external sphincter and opens to the skin. 3-Suprasphincteric-supralevator: It starts as intersphincteric and proceeds upwards and opens into the ischiorectal fossa and skin via puborectal or levator ani. 4-Extrasphincteric: Extends outside of all sphincters. 5-Submucous: Extends under the mucosa and opens to the skin [9].

American Gastroenterological Association (AGA) has suggested a binary category classification: i) simple and ii) complex. Simple fistulas are located at the inferior (superficial-submucous, inferior inter- or transsphincteric), with one outer orifice, no association with adjacent organs, no rectal stenosis or macroscopic proctitis. Complex fistulas are located at the superior (superior intersphincteric, suprasphincteric or extrasphincteric, high transsphincteric - those in the upper two thirds of the external sphincter) and there can be more than one external orifice. Presenting symptoms can be perianal discharge, discharge which is soiling the underwear, perianal mass, pain during and after defecation, anal bleeding, granulation around the outer hole, scarring, discoloration, and sometimes fever [10,13].

Pelvic magnetic resonance imaging and endoanal USG are used for clinical diagnosis of fistula and mapping of fistula. Treatment of perianal fistula varies according to the type and location of the fistula. Most commonly applied techniques are fistulotomy, fistulectomy, *LIFT* (Ligation of the Intersphinteric Tract), Glue and

Biological Plug Advancement flap procedures, seton applications, fistula tract laser closure (FiLaC™), video-assisted anal fistula treatment (VAAFT) and stem cell applications whose phase 3 clinical studies are ongoing. Aggressive surgical treatments are associated with high recovery rates. However, it is associated with an increased risk of sphincter damage and an increased risk of incontinence. On the other hand, relapse is more common in less aggressive methods [14,15].

More than a century has passed since the first definition of stem cells as ancestral cells of the germ line [16]. These cells may differentiate into several cell lines such as adipocytes, chondrocytes, osteoblasts and myoblasts in vitro [17]. Additionally, mesenchymal stem cells (MSCs) secrete various bioactive molecules that act in a paracrine manner to sustain angiogenic, antifibrotic, antiapoptotic and immunomodulatory responses in the target tissue, and can be used to regenerate damaged tissues [18]. Mesenchymal stem cells (MSCs) can be obtained from the bone marrow or adipose tissue. Adipose tissue is present in large quantities in most patients, MSCs can be easily harvested by a minimally invasive procedure, and provides a highly suitable population with an optimal differentiation potential, regardless of the age of the donor. The regenerative potential of MSCs derived from adipose tissue is similar to those reported in other tissues, as in 1 out of 100 adipose tissue cells are MSCs [19-22].

In our series, we used the Lipogems® system approved by the US Food and Drug Administration (FDA) to obtain MSC from adipose tissue [23].

In the pilot study, the mean age of the patients was 48 years. In our series it was 47. The mean operation time was 55 min, and it was 45 minutes in our series. Sixty percent of our patients had previously undergone fistula surgery. It was similar to the series in the literature. The most common postoperative complication was perianal abscess in our patients as in other series [11].

In the series in the literature, the fistula type was 60-70% transsphincteric and 20% extrasphincteric. The length of the fistula tract was found to be between 4-5 cm [24]. In our series, the most common type of fistula was transsphincteric and the length of the fistula tract was 4.45 cm. In the previously published series, complex perianal and rectovaginal fistulas of patients with inflammatory bowel disease were included, whereas none of our patients had inflammatory bowel disease.

In the series in the literature, before the application of autologous mesenchymal stem cells extracted from adipose tissue (ADSCs) to the fistula tract, the tract was curettage and the internal orifice was closed. We also used this technique in our study. In 2005, a Phase I clinical trial was initiated to examine the effect of ADSC transplantation on the repair of anal fistulas in Crohn's disease. In this study, 5 patients with Crohn's disease, and a total of 9 patients were treated and examined with weekly follow-up for 8 weeks (2 months) [25]. At the end, 6 fistulas (75%) were covered with epithelium. The phase 2 study was initiated by the same team to evaluate the safety and efficacy of autologous implantation of ADSCs (with or without fibrin glue) with 49 patients. In this study, 17 out of 25 patients (71%) (ADSCs with fibrin glue), and 4 out of 25 patients (16%) (fibrin glue only) showed fistula healing (P <0.001) (P <0.001). According to the same study, transplantation of autologous mesenchymal stem cells extracted from adipose tissue (ADSCs) in patients with suprasphincteric fistula was more effective than fibrin glue (p = 0.001). We did not use fibrin glue in our patients (4). Herreros et al. (2012) conducted a clinical phase III study and investigated the effects of autologous ADSC transplantation in the treatment of complex anal fistulas. In this study, 200 patients with complex anal fistula were treated. One-year results of the study with ADSCs (with or without fibrin glue) showed the best improvement in the group treated with ADSC (57.1%). There were no statistically significant differences between the ADSCs with fibrin glue (52.4%) and fibrin glue (37.3%) groups (p = 0.13) [26]. ADSCs (with or without fibrin glue) is suggested to be safe to use in the treatment of complex anal fistula.

The amount of transplanted ADSCs in the literature ranges from $20x10^6$-$120x10^6$ cells/ml. There is no consensus on how many sessions should be performed and co-administration with fibrin glue [27-29]. In our series, we applied 60x106 cells/ml for a single session and did not use fibrin glue. Most clinical studies have not reported a serious adverse event, so it can be concluded that this is a safe treatment, at least when used in adults [4,8,25]. We did not encounter any serious complications or side effects in our series.

It has been reported in the literature as a safe, minimally invasive procedure that reduces the length of stay and the risk of stool incontinence compared to the surgical option. The safety and efficacy of local therapies using ADSCs seem promising. The success rate is reported as 50-80% in the literature [12,28,30]. We achieved approximately 80% success rates in short-term follow-up.

In conclusion, in addition to surgical treatment of curettage of the fistula tract and closure of the inner mouth, autologous microfractured adipose tissue injection is a safe, feasible and reproducible procedure, and is an applicable method based on the early results of complex anal fistula healing. Limitations of our study were limited number of patients and its retrospective nature. Prospective studies with limited patient selection and large patient series are needed for routine use of Adipose-Derived Stem Cell Therapy in complex perianal fistula.

Riassunto

Si comunicano i primi risultati sulla sicurezza ed efficacia dell'iniezione di cellule staminali di derivazione adiposa (ADSC) insieme alla riparazione dell'orifizio interno per il trattamento della fistola perianale complessa.

Sono stati inclusi nello studio un totale di 10 pazienti, in prevalenza machi, sottoposti a iniezione autologa di cellule staminali di derivazione dal tessuto adiposo per il trattamento di fistola perianale complessa tra il dicembre 2017 e il gennaio 2018. Per preparare cellule staminali adipose micro frammentate autologhe è stato utilizzato il sistema Lipogems® approvato dalla FDA. Sono state valutate le caratteristiche demografiche, la storia della malattia infiammatoria intestinale, il tipo di fistola e la lunghezza del tratto di fistola determinati con ecografia endoanale, la durata media dell'operazione, le complicanze postoperatorie e la guarigione della fistola dei pazienti. La guarigione della fistola è stata valutata il 30°, 90° giorno e 9° mese.

L'età media dei pazienti era di 47 ± 13,1 e l'IMC media era di 28,3 ± 4,79. Nessuno dei pazienti aveva una malattia infiammatoria intestinale In base ai risultati ecografici endoanali, il tipo di fistola era transsfincterico, extrasincincico e soprasfincterico rispettivamente in 7, 2 e 1 paziente. La lunghezza calcolata del tratto di fistola basato sull'ecografia era 4,45 ± 1,69 [2,5-6,4]. La durata media dell'operazione è stata di 45 ± 7 minuti. Nessuno dei pazienti ha presentato tossicità correlata al trattamento, tuttavia, due pazienti hanno manifestato lividi nel sito di liposuzione. Il tasso di guarigione nel follow-up di 30 giorni, 90 giorni e 9 mesi è stato rispettivamente del 70%, 80% e 70%.

Si può concludere che oltre al trattamento chirurgico per curettage e chiusura del tratto di fistola, l'iniezione di tessuto adiposo micro frammentato autologo è una procedura sicura, fattibile e riproducibile che può essere eseguita sulla base dei primi risultati nella complessa guarigione della fistola anale.

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