Original Research Article

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Autologous bone marrow derived stem cells in the treatment of complex anal fistula

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ABSTRACT

Background: Treatment of anal fistula shows a great conflict between preservation of continence and eradication of sepsis. Stem cells extracted from bone marrow are able to home to inflamed tissues and repair injured ones. The aim of this study is to present the early results of injection of stem cells to tracts of complex recurrent fistulae. The primary outcome of the study is the healing of the complex fistula secondary outcomes include safety profile of the procedure.

Methods: 15 patients having complex anal fistulae. To prepare stem cells, bone marrow aspiration was performed under complete aseptic conditions. After several steps for isolation, viability testing and preparation, stem cells were suspended in sterile saline for injection in the patients. The internal opening was identified and closed then curettage of the tract with injection of cell suspension simultaneous with fibrin glue followed by injection of stem cell suspension around the tract and external opening finally it was closed. The first evaluation was performed after 8 weeks, a final evaluation after one year.

Results: Categorized into three groups (a) complete healing 8 patients (53.3%). (b) Incomplete healing 4 patients (26.7%) (c) Recurrence 3 patients (20%) No incontinence, abnormal tissue growth or major adverse effects were reported.

Conclusions: Use of autologous bone marrow-derived stem cells in anal fistula treatment is a recent modality with variable rates of success. Despite the rate of failure or incomplete healing but it still provides a solution to avoid continence impairment. It needs better evaluation with larger sample size, with long-term follow up. literatures.

Keywords: Autologous, Bone marrow, Complex anal fistula, Derived stem cells

INTRODUCTION

Anal fistulae, defined as abnormal connections between the rectum or anal canal and the surrounding perianal skin, are still problematic in daily colorectal surgical practice. When fistulae are treated by aggressive surgery it may result in high healing rates, however, this management is associated with the risk of sphincter damage and incontinence but if dealt with in a less aggressive way this may increase the danger of recurrence.¹ For the simple intersphincteric or transsphincteric low fistula surgical treatment such as fistulotomy or fistulectomy is mostly curative with a success rate reaching up to 100% cure rates. For complex, recurrent and branching fistulae minimally-invasive treatment such as fibrin sealants and pigcollagen plugs proved to be not highly successful. Although incontinence rates decreased recurrences increased. 3.4.5.6

Classically extracted from bone marrow (BM), mesenchymal stem cells (MSCs) were then found in

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many adult and fetal tissues. They are proven to home to inflamed tissues and to repair injured ones.^{7,8} The mesenchymal stem cells have multiple features including immunomodulation, releasing of growth factors and proangiogenic mediators, promoting and enhancing fibroblast migration to the wound, production of collagen as well as the power of differentiation.⁹⁻¹³

Based on this data, we evaluated the feasibility, safety, and efficacy of injection of autologous BM-derived MSCs in patients with complex anal fistulas a new modality in the treatment of these fistulae. The aim of this study is to present the early results of combined minimal surgical and cell therapy of complex recurrent fistulae. Stem cells were injected into this fistulous tract, to decrease the risk of incontinence associated with repeated attempts of surgical interventions. The primary outcome of the study is the healing of the complex fistula secondary outcomes includes feasibility and safety profile of the procedure.

METHODS

This cohort prospective study includes 15 patients suffering from complex anal fistulae that were admitted to the colorectal surgery unit. Faculty of medicine Cairo University from October 2014 to October 2016.Institutional Ethics Committee approval was obtained. All patients signed a detailed informed consent prior to any intervention.

Inclusion criteria were patients diagnosed with recurrent high trans-sphincteric anal fistula (Parks type II & Type III) of cryptoglandular origin. The fistula is considered complex when fistula tract under the perianal skin is nonpalpable during the physical examination, fistula tract is parallel to the rectum if examined with a probe multiple recurrences and presence of suprasphincteric tracts. Patients were excluded if they have Crohn's disease, rectovaginal fistula, pregnant or lactating woman, the patient has acute sepsis at the time of the intervention, and patients that need a surgical procedure for associated disease in the perianal region. 14,15

All patients underwent detailed history taking of their disease, complete general examination, and local examination in the form of rectal examination and proctoscopy, patients with any associated anorectal pathology other than fistula-in-ano were excluded from this study. Initial and post-treatment continence was assessed by Wexner score. ¹⁶

Usual preoperative laboratory investigations in the form of liver and kidney function tests, fasting blood sugar, complete blood picture and coagulation profile were performed. Specific investigations in the form of endoanal ultrasonography and magnetic resonance imaging (MRI) pelvic floor were done to assess the type of the fistula and to confirm the clinical diagnosis. Bone marrow aspiration was performed under complete aseptic

conditions using local anesthesia, bone marrow was aspirated from the posterior iliac crest, using a heparinfree preservative for withdrawal of 10-15 ml of bone marrow. Mononuclear cells layer was separated from heparinized bone marrow under complete aseptic conditions using Ficoll Hypaque by centrifugation at 1800 rpm for 20 minutes. Enumeration of CD34 and CD 44 positive cell population using the flow cytometry (Coulter Epics Elite flow cytometer).

Results were expressed as a percentage of cells expressing positive CD-34 and CD-44 within the gated population. Mononuclear cells were adjusted at 1 X 106 cells/ml & added to tissue culture flasks supplemented with fetal calf serum, modified media with glutamine and antibiotic with the total volume adjusted to 10 ml, incubated for 3 days at 37oC.Adherent cells were removed by pipettes and washed using Phosphate buffer saline. Trypan blue exclusion test was used for as a viability test. Isolated cells were subjected to immunophenotyping (CD 44 enumeration) and trilineage differentiation then stem cells were resuspended in sterile saline for injection in the patients.

Under general anesthesia without muscle relaxation to feel the muscle tone, patients were placed in lithotomy position. examination under anesthesia was performed to confirm the diagnosis. The internal opening was identified by injection of diluted methylene blue solution through the external opening closure of internal opening by absorbable suture 3/0. Deep Curettage of the tract with an injection of stem cell suspension simultaneous with fibrin glue (to act as a reservoir for the stem cells) inside the tract. Separate injection of stem cell suspension around the tract and the external opening. Closure of external opening with absorbable sutures. Operative time was calculated from the start of examination under anesthesia till closure of the external opening.

All the patients were kept for one day in the hospital to monitor for the immediate postoperative complications, Patients were discharged on oral antibiotics and analgesics (metronidazole and diclofenac Na) The follow-up visits were scheduled weekly for 8 weeks from the injection time, and then monthly for 10 months. The whole follows up period was one year

The first evaluation was performed after 8 weeks, and a final evaluation after one year from the procedure complete healing was defined as no purulent discharge from the external fistula orifice with a complete reepithelization with no recurrence for a year of follow-up. Partial healing was decreased suppuration or intermittent serous discharge. Non-healing or recurrence nothing improved and preoperative symptoms are the same as postoperative.

Safety was measured in terms of incontinence, serious adverse effects that need rehospitalization or changing the plan of treatment. Special care to the clinical assessment of the perianal region to screen for the appearance or growth of abnormal tissue, the persistence of or increased signs of suppuration, Quality of life scores at the 8th week and following one year of follow-up was estimated

Statistics were done by statistical package social science (SPSS) Version 12. For quantitative variables, descriptive analyses were expressed by using mean and standard deviation. The p-value <0.05 is statistically significant. Spearman's correlation was done between quantitative variables.

RESULTS

During the study period, autologous BM-MSCs combined with fibrin glue injected simultaneously was attempted in 15 cases of complex anal fistulae. Baseline characteristic of the study population was as follows the majority of the patients were males (13 patients 86.7%) while only two patients (13.3%) were females, the mean age of the patients was 38.56 ± 1.95 .

Table 1: Type of healing

Group	Frequency	Percent
Complete healing	8	53.3
Incomplete healing	4	26.7
Recurrence	3	20

All fistulae were recurrent with a recurrence from 3 to 6 times. Mean operative time was 16.33±2.28 minutes. Post bone marrow aspiration complications were in the form of pain that did not require analgesia in 7 (46.7%) cases or required analgesia " Oral and topical" in 7 (46.7%) cases, another complication was hematoma formation at the aspiration site. It was noted in one (7%) and it was conservatively treated with hot fomentations and topical and oral analgesics. No reported cases of sepsis or abscess formation at the aspiration site. The serous discharge was noted in all cases starting from 3rd to 5th week (mean 4th week) post injection.

Our findings were categorized into three groups Table (1), (a) Complete healing group including 8 patients (53.3%) where complete healing of the external opening was achieved and there was no any discharge. Ranging from the 12th week (90 days) and 4th month (120 days) "Mean 97.5000, SD 13.88730". (b) Incomplete healing group 4 patients (26.7%) where the external opening was closed with intermittent serous discharge and incomplete fistulous tract resolution. three patients report better quality of life and did not need 2^{nd} surgical intervention. In one case there was an evidence of fistula conversion into the sinus and Surgical coring for such sinus was done in a 2nd surgical intervention. (c) Recurrence group 3 patients (20%) where no healing of the external opening with continuous purulent discharge and no change in the preoperative symptoms. Recurrence was documented by reimaging by magnetic resonance imaging (MRI). No reported postoperative incontinence or abnormal tissue growth at fistula sites. No major adverse effects related to treatment were reported.

DISCUSSION

Surgical treatment of complex anal fistula may result in incontinence and recurrences that may need multiple surgeries. To minimize the effect of treatment on the sphincter complex many treatment modalities have been evolving such as plugs, fibrin glue and recently use of autologous bone marrow MSC. To our knowledge, this is the first study in Cairo University faculty of medicine on treatment of recurrent and complex anal fistula using autologous bone marrow-derived mesenchymal stem cells.

In the study population 15 patients with complex recurrent anal fistulae, more than the half of patients 53.3% (8 patients) achieved a complete cure for their disease with no effect on continence at all. While 26.7% of patients (4 patients) had continuous intermittent serous discharge with periods of external opening closure. Even in those percent of cases where the fistula did not heal completely, patients reported significant improvement in the quality of life compared with pre-injection one. Three patients (20%) the serous discharge turned into continuous purulent discharge with no improvement of the pre-injection symptoms which represent the recurrence group. Fibrin glue is used for the treatment of anal fistula, while early results were encouraging but later on, the data showed that the initial high rates of success 74% were not actually the same after longer follow. It reached as low as 14%. 17,18 The evolving role of stem cells in medical practice allowed multiple studies to use mesenchymal stem cells with variable study designs. In all of the studies conducted till now, surgery was combined with cell application as a single- step procedure, with or without fibrin glue reaching the healing rate of 50%-90% for fistulae. 19,20 In the current study results obtained are due to the use of MSC. We used fibrin glue for injection to act as a reservoir for MSC, so it could act for a longer time.²¹ This is supported by Garcia-Olmo D et al., who reported that healing is more than four times higher in patients treated with MSC plus fibrin glue than in those treated with fibrin glue alone.22

Bone marrow-derived MSC has a unique biological behavior through the ability to differentiate.²³ On basis of our findings, MSCs injected into the inflamed tracts will induce healing and suppress inflammation leading to the closure of the inflamed tracts.

Regarding the safety profile, this cell being extracted from the same patient own body safety problems regarding the transmission of blood born infections where are not anticipated.²⁴ No major adverse events related specifically to the procedure happened in our study. The risk of neoplastic development is of concern. This needs a

longer follow up period yet published data with longer periods of follow up and multiple biopsies taken revealed no evidence of neoplastic changes.²⁵

The current study showed no change post-MSCs Injection in Wexner incontinence score, this was nearly anticipated as the curettage and the injection does not interfere with the integrity of the anal sphincters musculature and also no extensive fibrosis as in fistulotomy or Seton application. Surgical interference in complex fistula always carries the risk of incontinence.

Garcia-Olmo D et al., reported that the cost of therapy with stem cells is difficult to ascertain, the cost of production was in the range of 12000\$-18000\$ to treat 24 patients. However, this estimate corresponds to an experimental production cost. ²⁶ In the present study, the costs of the MSC Aspiration and isolation and the injection process under anesthesia are still more than the cost of the surgical intervention alone and it was one of the major obstacles for expanding the application of such study in a large number of patients. AS every new technology, industry and spread could markedly decrease the costs.

CONCLUSION

The use of autologous bone marrow-derived stem cells in the treatment of the anal fistula is a recent treatment modality for the problematic complex anal fistula with a variable rate of success between studies. Despite the rate of failure or incomplete healing but it still provides a solution to avoid continence impairment and extensive tissue scarring which follows the ordinary surgical procedures, which still carry the risk of failure rate. This technique needs to be expanded to larger patients sample size, with the inclusion of more types of fistulae (Crohn's and rectovaginal), with long-term follow up to assess the efficacy and safety.

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Ethical approval: The study was approved by the

Institutional Ethics Committee

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