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The Impact of Autologous Bone Marrow Mesenchymal Stem Cells (MSCs) in the Treatment of Stress Urinary Incontinence: Short term Follow-up

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Abstract

Objective : The aim of this study is to assess the effectiveness and tolerability of autologous stem cell injection for the treatment of female stress urinary incontinence.

Patients and Methods: This was a prospective study which involved 12 women with stress urinary incontinence. One hundred and twenty milliliters of bone marrow were aspirated under aseptic conditions from posterior superior iliac spine. MSCs were separated using plastic adherence and verification of the cell population done by morphology, immunophenotyping and trilineage differentiation potential. Patients received transurethral endoscopic or periurethral ultrasonography-guided injections of autologous bone marrow MSCs. Patients were followed up using voiding diaries, trans-vaginal ultrasonography, urodynamics and quality of life questionnaire over a 3 months period.

Results: The objective cure rate in these 12 patients was 85% subjective complete cure rate was 66.67% (8 patients), 2 patients improved partially (16.7%), 2 patients did not improve (16.7%). Overall mean increase in the bulk of sphincter as measured by transvaginal ultrasonography 3 months post stem cell injection was 15.39% which forms about 1/7 of the sphincter tissue bulk.

Conclusion: Autologous bone marrow derived MSC injection is a simple, safe and effective procedure for treating stress urinary incontinence. The short term results of the procedure show a success rate similar to that of the standard techniques; without their disadvantages in terms of complications especially voiding dysfunction. Further studies with long-term follow up are needed to assess the integrity of the results.

Keywords: Autologous bone marrow, Mesenchymal stem cells, Stress Urinary incontinence, Stem cell therapy.

Introduction

Stress urinary incontinence (SUI) is the involuntary loss of urine from the urethra during coughing, sneezing or physical exertion [1]. It is the most common form of urinary incontinence in women affecting almost 25% of women [2], with huge impact on the social and psychological state of patients. The pathogenesis of SUI is complex with physical trauma and abnormalities in extracellular matrix as possible causes. Also, genetic predisposition has been suggested. Current therapeutic modalities are diverse and none of which are satisfactory. Conservative management in the form of behavioral therapy, pelvic floor exercises and pharmacologic therapy are effective only in mild symptoms. Moreover, they are symptomatic and do not address the underlying pathology [3,4].

Surgical interventions aim at correcting underlying defect through sling procedures, artificial sphincter, and injectable bulking agents [5,6]. Despite advances in these current lines of therapy, the prognosis remains below expectations. Regenerative medicine is a new branch of medicine that aims to restore function or alter biological state of the organ [7]. Regenerative medicine may use cell-based approaches or biomaterial injection. A number of cell-based approaches have been tackled by researchers in SUI; namely, chondrocyte injection, muscle cell injection, or pluripotent stem cells of embryonic, adipose or mesenchymal origin [8]. To our knowledge, this is the first clinical trial to evaluate the safety and short term efficacy of autologous mesenchymal stem cell therapy in cases of SUI.

Patients and Methods

This prospective study involved twelve women complaining of SUI. This study was carried out in the period from January 2014 till December 2015. Explanation of the procedure and written consent was taken for every patient recruited in this study.

Inclusion criteria

1. Adult females (≥ 21 years)
2. Informed written consent
3. Clinical or urodynamic diagnosis of SUI
4. Stress urinary incontinence with failed medical treatment
5. No concomitant diseases
6. No anticholinergic medication treatment as "Detrusitol" or

"Detrupan"

7. Negative pregnancy test

Exclusion criteria

a. Medically treatable or controlled incontinence

b. Pregnant females or immediately postpartum

c. Urge incontinence

d. Other concomitant diseases

e. History of spinal, pelvic or urinary tract trauma that may affect bladder function (including bladder stones in the last six months)

f. Cessation of anticholinergic medication in the last 21 days before participation in the study

g. History or recent diagnosis of bladder cancer

h. Any genitourinary tract infection within the last 4 weeks

i. Taking any antiplatelet medication or any drug that increase the bleeding tendency within the 3 days preceding the injection

j. History of hemophilia or other diseases affecting blood coagulation

k. Known hypersensitivity to the anaesthetic drugs as lidocaine

l. Neurological disease affecting the motor system

All patients were subjected to

1. Full history taking, complete physical and neurological examination

2. Voiding diary

3. Urodynamic study which included filling cystometry, voiding cystometry and pressure flow studiess

4. Trans-vaginal ultrasonography

5. Completion of quality of life questionnaire

6. Counseling and written consent was taken from every patient

7. Clinically, the type of incontinence was defined as urodynamic stress incontinence in 10 patients (83.3%) and mixed incontinence in 2 patients (16.7%)

Stem cells retrieval, culturing and processing

i. 120 milliliters of bone marrow were aspirated from the posterior superior iliac spine under local anesthesia

ii. Mononuclear cells (MNCs) were isolated by density gradient centrifugation using Ficol/Hypaque

iii. MSCs were isolated through plastic adherence by the seeding of MNCs in T25 tissue culture flasks in complete culture media (DMEM, 10% antibiotics/antifungal, 20% autologous serum). Flasks are incubated at 37˚C in CO2 incubator for 3-5 days

iv. After 5 days, flasks were evaluated under inverted microscope for the number and morphology of MSCs. Non-adherent cells were discarded and medium replenished

v. Half of the medium was changed every 3 days

vi. Flasks were evaluated until cells reach about 80-90% confluence

vii. MSCs were harvested using Trypsin-EDTA (0.25%) for 5 minutes. Afterwards, trypsin action was stopped by the addition of autologous serum

viii. MSCs were washed using phosphate buffered saline (PBS), counted, tested for viability using trypan blue exclusion test

ix. MSC were identified using immunophenotypic markers (positive for CD90, CD105, CD271, CD44 and negative for CD45 and CD34)

x. Cells were suspended in sterile saline, a minimum of 2X106 MSCs cells in a 10 cc syringe, are periurethrally injected with trans-vaginal ultrasonography guidance or by endoscopy, in the region of the external urethral sphincter. Quality control check for the sterility by the performance of bacterial aerobic and anerobic cultures was done to ensure the complete aseptic conditions during the specimen retrieval, preparation, storage, and injection

Injection procedure

i. Stem cell injection was done under transvaginal ultrasonography guidance in 10 cases under local anesthesia and by urethrocystoscopy in 2 cases under spinal anesthesia

ii. Sterilization and draping of the field (from umbilicus to midthigh) with special consideration to introitus

iii. Urethral catheter, Foley's 16F was applied in cases performed under transvaginal ultrasound guidance to help assessing the bladder neck and presumed site of rhabdosphincter for proper injection of stem cells

iv. Accessing the periurethra by a needle of a 10 cc syringe was done under transvaginal utlrasonography guidance at 3,9,12 o'clock, injecting around 3.5 cc in each port, at the presumed site of rhabdosphincter as it appears by transvaginal ultrasound

v. In the 2 cases performed by urethrocystoscopy, we used the 22 F. sheath, and injection was done directly at the site of rhabdosphincter at 3,9,12 o'clock also, using a semi flexible 9F needle catheter

vi. 3.5 milliliters were injected at each of the three sites (3, 9, 12 o'clock)

vii. No postoperative urethral catheters were applied

viii. It was a one day procedure, all patients were discharged in the same day

Postoperative care

i. Prophylactic oral antibiotics and oral analgesics were prescribed to patients and were advised to avoid heavy lifting, exercise and sexual intercourse for 4 weeks

ii. Postoperative assessment included patients' interview, subjective assessment of improvement, PVR, voiding diary, transvaginal ultrasonography to assess the thickness of both urethra and rhabdosphincter, assessment of continence grade and a patient questionnaire

iii. Patients were scheduled for 4 visits, dating 1st, 2nd, 4th week post- stem cell injection and 3rd month visit

Statistical analysis

Retrieved data were recorded on an investigative report form. The data were analyzed with SPSS® for Windows®, version 15.0

(SPSS, Inc, USA). Description of quantitative (numerical) variables was performed in form of mean, standard deviation (SD) and range. Description of qualitative (categorical) data was performed in the form of numbers and percent. Analysis of numerical variables was performed by using student's unpaired t-test (for two groups) or ANOVA (for more than two groups). Significance level was set at 0.05.

Results

It was a prospective study conducted on 12 women with stress urinary incontinence. The objective cure rate in the 12 patients was 85% by cough stress test and no urine leakage during urodynamic study with increased intra-abdominal pressure while the subjective level of complete and partial patient satisfaction was 66.67% (8 patients) and 16.7% (2 patients) respectively. Complete patient satisfaction means no loss of urine under stress, no voiding difficulty, any urinary symptoms at filling. Partial patient satisfaction means that patient had periodic or rare episodes of dribbling at stress but considered themselves subjectively cured because incontinence occurred under extreme stress and no pads were required on a daily basis, two of the 12 patients (those with partial improvement) had multiple prior procedures for the treatment of SUI. Only two patients were unimproved (16.7% of cases).

Table 1 and Figure 1 show an improvement in urethral pressure profilometry regarding the urethral closure pressure and functional urethral length.

Trans-vaginal ultrasonography before and after stem cell injection proved that there is a gradual increase in the bulk of the external sphincter "readings were taken 5 mm and 10 mm distal to bladder neck" along its 2 dimensions (anterior and posterior dimensions), a gradual increase in the bulk of the sphincter was noted (Table 2, 3 and Figure 2).

Table 1: Comparison of urodynamic variables of urethral pressure profile in the 12 patients with SUI pre and post injection

Variable	Pre	Post
Urethral Closure pressure (cm H_2O)	19±5.4	30±4.3
Functional urethral length (mm)	20±3.3	90±20.7

Values are given as mean ±SD

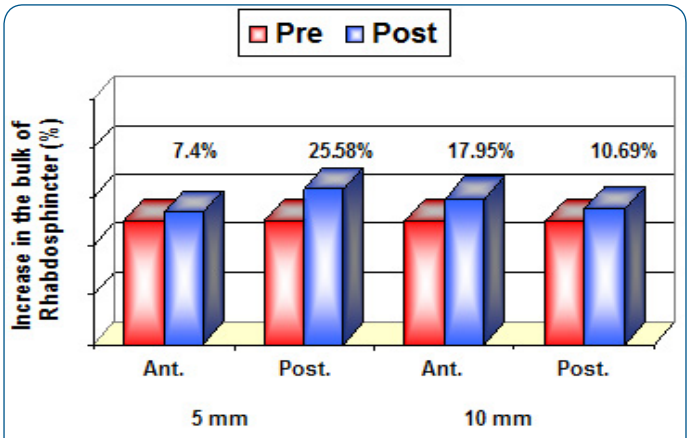

Figure 1: Comparison of urodynamic study variables of urethral pressureprofile in the 12 patients with SUI pre and post injection

Summation of these results divided by number of readings gives us the overall mean increase in the bulk of sphincter post stem cell injection, which is 15.39% which means that stem cell injection builds around 1/7th of the sphincter tissue bulk again in 3 months, as shown in Figure 2.

Table 2: Comparison between the anterior and posterior dimensions of rhabdosphincter being taken 5 mm and 10 mm distal to the bladder neck, pre and post injection

Time Distal to bladder neck	Pre		1 week after		After 3 months	
	Post	Ant.	Post	Ant.	Post.	Ant.
5 mm	4.3	4.6	5.2	4.45	5.4	5
10 mm	4.7	3.9	4.25	4.5	5.2	4.6

Table 3: Mean increase in the bulk of the sphincter as taken 5 mm and 10 mm distal to bladder neck

	5 mm		10 mm	
	Post.	Ant.	Post.	Ant.
Net increase in the bulk of sphincter	25.58%	7.4%	10.64%	17.95%

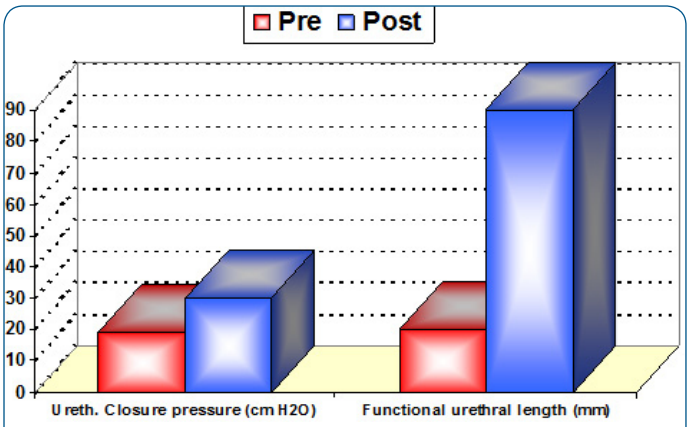

Figure 2: Mean increase in the bulk of the sphincter as taken 5 mm and 10 mm distal to bladder neck

Discussion

SUI is a disorder involving involuntary leakage of urine with increased intra-abdominal pressure as during coughing or sneezing. It has a major detrimental effect on the medical and psychological quality of life of patients [9]. Effective closure of the urethra depends on the combined actions of pelvic floor muscles and urethral sphincter [10]. The pathophysiology of SUI is complex; Wen et al [11]demonstrated abnormal proteoglycan expression mediated by ovarian hormones in the extracellular matrix of pelvic floor muscles in women with SUI which may contribute to the pathogenesis of SUI [11]. Genetic predisposition was also suggested; Chen et al examined the differential expression of the genes involved in extracellular matrix metabolism and demonstrated alteration in genes involved in elastin metabolism pathway [12]. Wen et al also demonstrated altered transforming growth factor beta expression in the vaginal wall fibroblasts of women with SUI [13].

Treatment options for cases of SUI are surgical, medical, and behavioral in the form of pelvic floor exercises and electric stimulation of the pelvic floor muscles. Additionally, vaginal inserts can be used in cases prolapse. Medical treatment in the form of estrogen replacement is questionable [14]. Current gold standard in the treatment of SUI is the suburethral sling operation in a trial to reinforce the levator ani muscle and the supportive ligaments.

Regenerative medicine represents the new era in medicine, allowing for the first time to rebuild damaged tissues. Formation of new tissues needs the presence of cells, matrix or scaffolds and microenvironment [8]. Cellular options for regeneration in urinary incontinence are variable. Early trials used autologous chondrocytes making use of their ability to produce extracellular matrix. Chondrocytes are easily isolated from porcine ears and expanded in culture. Chondrocytes were injected endoscopically at the vesico-ureteral junction. The results were promising with 50% of patients cured for 12 months after a single injection. The main limitation of this study was the source of chondrocytes [15-17].

Using myoblasts for SUI has been thoroughly investigated. Myoblasts were demonstrated to survive and function in the urethral sphincter in animal models [18]. Myogenic progenitor cells were used as they can proliferate and form myotubules and new muscle tissue. One of the important clinical trials reported the use of autologous myoblasts and fibroblasts through transurethral ultrasound-guided injections. Follow-up showed 38 out of 42 women completely continent. However, this report was later retracted from Lancet [19].

Stem cells were proposed as a source of repair of sphincter muscle. Stem cells are characterized by their ability to self-renew, proliferate and differentiate into many cell types according to the microenvironment they are implanted in [20]. Early trials with stem cells used embryonic stem cells. However, their clinical applications were not allowed due their liability to form ethical controversies [21]. Adult stem cells are more practical candidates for regenerative trials with no ethical concerns. Mesenchymal stem cells have many advantages in this setting; namely, easy isolation, high proliferative potential and efficient plasticity in vivo. They can be derived from adipose tissue, synovial membrane or bone marrow [22]. A number of workers used adipose-derived MSCs in-vitro for myogenic differentiation [23] and in experimental animal models [24]. Bone marrow derived MSCs were induced for myogenic differentiation in culture, this makes them a practical candidate for cell therapy [25].

To our knowledge, this is the first clinical trial using autologous bone marrow derived injected intraurethral through transvaginal sonographic guidance. This trial was completely safe, with no complications over the follow-up period. Complete stoppage of dribbling was seen in 66.67% of the patients, and partial improvement was seen in 16.7%. However, urodynamic study with increased intra-abdominal pressure showed no urine leakage in 85% of patients. Increased sphincter bulk was seen in all cases with a mean of 15% increase. These findings are comparable to those of the myogenic progenitor cell trial [19]. Moreover, MSCs are easier in acquisition, isolation and expansion.

Conclusion & Recommendations

In view of such findings, stem cell injection is a simple, effective, and safe procedure for treating stress urinary incontinence. The short term results for this technique showed a success rate similar to that of the most effective techniques, without their disadvantages in terms of complications, particularly voiding dysfunction. However, further studies and longer term follow up are needed to confirm that this will be the standard form of treatment for stress urinary incontinence in the future, and to assess the duration of results.

References

1. Klauser A, Frauscher F, Strasser H, Helweg G, Kolle D, Strohmeyer D, et al. Age-related rhabosphincter function in female urinary stress incontinence. J Ultrasound Med. 2004; 23(5):631-637.
2. Hannestad YS, Rortveit G, Sandvik H, Hunskaar S. A community-based epidemiological survey of female urinary incontinence: the Norwegian EPINCONT study. Epidemiology of Incontinence in the County of Nord-Trondelag. J Clin Epidemiol. 2000; 53(11):1150–1157.
3. Alhasso A, Glazener CM, Pickard R, N'Dow J. Adrenergic drugs for urinary incontinence in adults. Cochrane Database Syst Rev. 2003; 2:CD001842.
4. Bo K, Talseth T, Holme I. Single blind, randomised controlled trial of pelvic floor exercises, electrical stimulation, vaginal cones, and no treatment in management of genuine stress incontinence in women. BMJ. 1999; 318(7182):487–493.
5. Sharifi-Aghdas F. Surgical management of stress urinary incontinence. Urol J. 2005; 2:175–182.
6. Kuhn A, Gelman W, Kuhn P. Injectable therapy for urinary incontinence: a review. Praxis. 2004; 93(6):188–192.
7. Atala A. Tissue engineering and regenerative medicine: concepts for clinical application. Rejuvenation Res. 2004; 7(1):15–31.
8. Bae JH, Yoo JJ. Cell-Based Therapy for urinary incontinence. Korean J Urol. 2010; 51(1):1-7. doi: 10.4111/kju.2010.51.1.1.
9. Romanzi LJ. Urinary incontinence in women and men. J. Gend Specif Med. 2001; 4:14-20.
10. Liu X, Zhao Y, Pawlyk B, Damaser M, Li T. Failure of elastic fiber homeostasis leads to pelvic floor disorders. Am J Pathol. 2006; 168(2):519-528.
11. Wen Y, Zhao Y, Li S, Polan M, Chen B. Differences in mRNA and protein expression of small proteoglycans in vaginal wall tissue from women with and without stress urinary incontinence. Hum Reprod. 2007; 22(6):1718-1724.
12. Chen B, Wen Y, Zhang Z, Guo Y, Warrington J, Polan M. Microarray analysis of differentially expressed genes in vaginal tissues from women with stress urinary incontinence compared with asymptomatic women. Hum Reprod. 2006; 21(1):22-29.
13. Wen Y, Polan M, Chen B. Do extracellular matrix protein expressions change with cyclic reproductive hormones in pelvic connective tissue from women with stress urinary incontinence? Hum Reprod. 2006; 21(5):1266-1273.
14. Richer H, Burgio K, Goode P, Borello-France D, Bradley C, Brubaker L, et al. Non-surgical management of stress urinary incontinence: ambulatory treatments for leakage associated with stress (ATLAS) trial. Clin Trials. 2007; 4(1):92-101.
15. Bent AE, Tutrone RT, McLennan MT, Lloyd LK, Kennelly MJ, Badlani G. Treatment of intrinsic sphincter deficiency using autologous ear chondrocytes as a bulking agent. Neurourol Urodyn. 2001; 20(2):157–165.

16. Atala A, Kim W, Paige KT, Vacanti CA, Retik AB. Endoscopic treatment of vesicoureteral reflux with a chondrocyte-alginate suspension. J Urol. 1994; 152 (2 Pt 2):641–643.

17. Diamond DA, Caldamone AA. Endoscopic correction of vesicoureteral reflux in children using autologous chondrocytes: preliminary results. J Urol. 1999; 162(3 Pt 2):1185–1188.

18. Strasser H, Berjukow S, Marksteiner R, Margreiter E, Hering S, Bartsch G, et al. Stem cell therapy for urinary stress incontinence. Exp Gerontol. 2004; 39(9):1259–1265.

19. Kleinert S, Horton R. Retraction--autologous myoblasts and fibroblasts versus collagen [corrected] for treatment of stress urinary incontinence in women: a [corrected] randomised controlled trial. Lancet. 2008; 372(9641):789-90. doi: 10.1016/S0140-6736(08)61320-3.

20. Keller G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. Genes Dev. 2005; 19(10):1129–1155.

21. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol. 2000; 18(4):399–404.

22. Drost AC, Weng S, Feil G, Schäfer J, Baumann S, Kanz L, et al. In vitro myogenic differentiation of human bone marrow-derived mesenchymal stem cells as a potential treatment for urethral sphincter muscle repair. Ann N Y Acad Sci. 2009; 1176:135–143. doi: 10.1111/j.1749-6632.2009.04610.x.

23. Jack GS, Almeida FG, Zhang R, Alfonso ZC, Zuk PA, Rodriguez LV. Processed lipoaspirate cells for tissue engineering of the lower urinary tract: implications for the treatment of stress urinary incontinence and bladder reconstruction. J Urol. 2005; 174(5):2041–2045.

24. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001; 7(2):211–228.

25. Lin G, Wang G, Banie L, Ning H, Shindel AW, Fandel TM, et al. Treatment of stress urinary incontinence with adipose tissue-derived stem cells. Cytotherapy. 2010; 12(1):88-95. doi: 10.3109/14653240903350265.