Intramuscular Injection of Adipose Tissue Derived Stromal Vascular Fraction in Subjects with Poliomyelitis: Case Reports


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Abstract
Poliomyelitis is a disabling condition that cause muscular weakness, and may develop further deterioration known as post-polio syndrome a few decades later. Adipose tissue derived stromal vascular fraction is a major source of mesenchymal stem cells and its clinical application for neuromuscular disease has been advocated. Four male participants with lower limb weakness and gait disturbance secondary to poliomyelitis underwent liposuction procedure. Stromal cells freshly isolated from the adipose tissue extract were injected into affected muscles. Liposuction and intramuscular injection procedures were done without any significant adverse event in all participants. Stromal cells were successfully isolated and showed sufficient cell counts and colony forming units. Participants reported improved walking ability, increased muscle strength, decreased fatigue and general well-being sense. We found possibility of improving muscle function in poliomyelitis subjects by intramuscular injection of autologous adipose tissue derived mesenchymal stromal cells, and call for further research.

Keywords: Mesenchymal stromal cells; Adipose tissue; Poliomyelitis.

Introduction
Stem cell transplantation is one of the emerging treatments that are expected to improve permanent physical impairment among persons with disabilities. For those with muscular weakness, several cell lines have been proposed as having myogenic potential, which include satellite cells, muscle-derived stem cells, mesoangioblasts, and mesenchymal stem cells [1]. Human adipose tissue is one major source of mesenchymal stem cells. The stromal vascular fraction (SVF) extracted from adipose tissue contains rich of such cells and has been found to be feasible for clinical use [2].

Poliomyelitis is a viral infection that affects motor neurons and causes permanent muscle weakness. Though polio has been eradicated in the developed world owing to the introduction of vaccine, many of aged survivors suffer from further deterioration of motor function which is called post-polio syndrome. There are no restorative treatments for this condition so far except applying orthotics and assistive devices to maintain activity [3]. Because of the debilitating nature of the disease and impending health situation of the polio survivors who are getting older, claim for use of stem cell therapy for this population was raised [4]. The target for stem cell could be either nerve cell or muscle. Adipose tissue-derived stem cells have been investigated for the treatment of skeletal muscle injury [5] and motor neuron disease [6]. However, no trials of stem cell therapy for poliomyelitis have been reported yet.

We assumed that intramuscular injection of SVF derived from autologous adipose tissue might enhance muscle function of poliomyelitis subjects by either myogenic or paracrine effect of mesenchymal stem cells in the SVF. In this brief report, we present four participants who complained of gait disturbance secondary to poliomyelitis since childhood, and had liposuction and intramuscular injection of freshly isolated SVF.

Methods
Four male participants with poliomyelitis were enrolled in the study. This study was approved by the Institutional Review Board and written informed consent was obtained from all participants before enrolment.

Intervention
Extraction of adipose stem cells and intramuscular transplantation
Under general anesthesia, liposuction of abdominal subcutaneous fat was performed. Tumescent fluid was made
with normal saline 1,000 ml, 2% lidocaine and 1 ml of 1:1,000 epinephrine, and was infiltrated into subcutaneous fat layers of abdominal wall. And then liposuction was performed and cells were isolated from laboratory. Isolation procedure is described below. Cells were diluted with normal saline and injected directly into the affected limb muscles.

Isolation for Stromal Vascular Fraction (SVF)

We followed manufacturer’s guidance for the SVF isolation using SmartX kit (Dongkoo bio & pharma Co., Seoul, South Korea) by enzymatic digestion method. Briefly, adipose tissue was centrifuged at 3,000 rpm for 5 minutes to remove water, tumescent solution and oil. The 50 ml of fat tissue digested with 0.075% collagenase type 1 at 37°C for 30 minutes under gentle agitation. After the digested tissue was filtered through 75 μm strainer to remove residual tissue, the cell suspension was centrifuged and washed with phosphate buffered saline three times at 3,000 rpm for 3 minutes. Total and live cell counts were performed using the nucleocounter® NC-200TM automated cell counter (Chemometec, Denmark).

Colonies forming unit assay for Stromal Vascular Fraction (SVF)

Freshly prepared SVF were re-suspended in growth medium, and plated in 35 mm dishes at densities of 5 × 10^3 for P0 in 2 ml of growth medium. The medium was changed every 3–4 days. Three weeks after plating, cells were stained with 3% crystal violet (Tech-innovation, South Korea) in methanol. The staining solution was discarded and the dishes observed under a microscope. A collection of cells of around 100 mm^2 (~ 50 cells) was counted as one colony.

Results

Case 1

A 56-year-old male subject participated in the study. He has been suffering from poliomyelitis sequelae since he was 3 years old. Both of his lower limbs were affected by poliomyelitis, more severely in the left side. He could walk independently without brace until 10 years previously when he began to wear left knee-ankle-foot orthosis for his left leg for walking. Without the orthosis, he had to use bilateral crutches. His weakness eventually led him to use wheelchair more frequently.

Liposuction procedure was performed without producing any significant adverse events. From the adipose tissue extract of 35 ml, 1.74 × 10^7 stromal cells (viability 63%) were isolated. The number of colony forming units was five. Cells were diluted with 20 ml normal saline and injected into bilateral rectus femoris, gluteus medius, adductor magnus and biceps femoris muscles.

After he had intramuscular SVF injection, he became to be able to walk without crutches. He reported that his lower limb muscle strength was improved after the procedure from 20% to 30% of normal in his subjective sense. Objective clinical evaluation was not performed for this case.

A few months later, he was still able to walk with a single cane.

Case 2

A 60-year-old male subject participated in the study. He has been suffering from poliomyelitis sequelae since he was 3 years old. Both of his lower limbs were affected by poliomyelitis, more severely in the left side. He could walk independently without brace until 10 years previously when he began to wear left knee-ankle-foot orthosis for his left leg for walking. Without the orthosis, he had to use bilateral crutches. His weakness eventually led him to use wheelchair more frequently.

Liposuction procedure was performed without producing any significant adverse events. From the adipose tissue extract of 35 ml, 1.74 × 10^7 stromal cells (viability 63%) were isolated. The number of colony forming units was five. Cells were diluted with 20 ml normal saline and injected into bilateral rectus femoris, gluteus medius, adductor magnus and biceps femoris muscles.

After he had intramuscular SVF injection, he became to be able to walk without crutches. He reported that his lower limb muscle strength was improved after the procedure from 20% to 30% of normal in his subjective sense. Objective clinical evaluation was not performed for this case.

A few months later, he was still able to walk with a single cane.

Case 3

A 54-year-old male subject participated in the study. He has been suffering from poliomyelitis sequelae since he was 4 years old. All 4 limbs were affected by poliomyelitis, more severely in both lower and left upper extremities. He denied other past medical history except surgical treatment for right ankle fracture 3 years previously. The manual muscle test revealed MRC grade 1–3 in the left upper extremity, 4–5 in the right upper extremity, 3–4 in both hip and knee, and 1–2 in both ankle. He could walk independently without walking aid, but showed trunk tilting during stance phase, more markedly toward left side, and foot drop during swing phase bilaterally.

Liposuction procedure was performed without producing any significant adverse events. From the adipose tissue extract of 50 ml, 6.07 × 10^7 stromal cells (viability 72%) were isolated (Figure 1). The number of colony forming units was eighteen. Cells were diluted with 24 ml normal saline and injected into bilateral gluteus medius, rectus femoris, biceps femoris, right biceps brachii, triceps brachii, tibialis anterior and gastrocnemius muscles.

After the injection, he reported that his muscle strength was increased and general well-being sense improved. He also reported that he felt increased muscle strength when walking as well as driving a car.

For objective outcome evaluation, muscle strength, physical performance and skeletal muscle mass were measured before treatment, 2 weeks, 1 month, 2 months, and 3 months after treatment.
Muscle strength was measured by hand-held dynamometer. Most of muscle groups injected with SVF showed increasing tendency in strength. For example, left hip extensor muscle showed steady increase in strength after injection, from 92N to 145, 140, 149, and 149 N at 2 weeks, 1 month, 2 months, and 3 months after injection respectively (Figure 2A). Both ankle dorsiflexors also started to gain strength after 2 weeks of injection. Especially, left ankle dorsiflexor recorded 0N before treatment but showed some strength after injection (Fig. 2B). Despite these findings, the subject complained of muscle fatigue after he performed excessive leg press exercise in order to maximize strengthening effect. Muscle fatigue resulted in decreased knee extensor strength, and it was recovered a couple of months later.

Physical performance was measured by Short Physical Performance Battery (SPPB) [7], which consists of balance test, gait speed test, and chair stand test. The SPPB recorded full score at both before and after treatment, however, gait speed showed increasing trend from 3.75 sec/4 meter walk to 3.66, 3.21, 2.97, and 3.01 at 2 weeks, 1 month, 2 months, and 3 months after injection respectively.

Lean body mass measured by dual x-ray absorptiometry did not show notable changes.

Case 4

A 56-year-old male subject participated in the trial. He has been suffering from poliomyelitis sequelae since he was 3 years old, and his right lower limb was affected. The manual muscle test revealed MRC grade 2 (knee and ankle) to 3 (hip) in the right lower extremity. He could walk independently without walking aid, but showed trunk tilting toward right side during stance phase, insufficient knee flexion and mild foot drop during swing phase.

Liposuction procedure was performed without producing any significant adverse events. From the adipose tissue extract of 100ml, 3.15x10^7 stromal cells (viability 80%) were isolated. The number of colony forming unit was three. Cells were diluted with 20ml normal saline and injected into right rectus femoris and gluteus medius muscles.

After the injection, he denied any subjectively notable changes. He didn't report any feeling of increased strength or vitality.

For objective outcome evaluation, muscle strength, physical performance, skeletal muscle mass, and quality of life were...
measured before treatment, 2 weeks, 1 month, 2 months, 3 months and 6 months after treatment.

Muscle strength measured by hand-held dynamometer did not show consistent changes. Physical performance measured by SPPB recorded 9(balance test 2, gait speed test 3, and chair stand test 4) before treatment and increased to 11(balance test 4, gait speed test 3, and chair stand test 4) after treatment. Walking ability was measured by 10 meter walking test and 6 minutes walking test. Comfortable walking speed measured during 10 meter walking test increased from 0.66 m/s before treatment to 0.74, 0.84, and 0.97 m/s at 2 weeks, 3 months, and 6 months after injection respectively, while fast walking speed measured during 6 minute walking test showed only slight increase from 1.00 m/s to 1.03 m/s after treatment (Fig. 2C).

Lean body mass measured by dual x-ray absorptiometry did not show consistent changes. Health-related quality of life measured by WHO quality of life-BREF did not show notable changes either in all domains.

The findings of each case are summarized in Table 1.

### Table 1: Summary of findings

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Affected limb</th>
<th>Adipose tissue extract</th>
<th>SVF cells: Total /Live</th>
<th>CFU</th>
<th>Number of muscles injected</th>
<th>Adverse events</th>
<th>Subjective report of treatment effect</th>
<th>Objective clinical outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>56</td>
<td>Right lower limb</td>
<td>50ml</td>
<td>1.09x10^7</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>Walking ability improved Fatigue decreased General well-being sense</td>
<td>Not available</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>60</td>
<td>Both lower limbs</td>
<td>100ml</td>
<td>1.74x10^7</td>
<td>5</td>
<td>8</td>
<td>None</td>
<td>Walking ability improved Muscle strength increased</td>
<td>Not available</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>54</td>
<td>Both upper and lower limbs</td>
<td>100ml</td>
<td>6.07x10^7</td>
<td>18</td>
<td>10</td>
<td>None</td>
<td>Muscle strength increased General well-being sense</td>
<td>Muscle strength increased (dynamometer) Gait speed increased</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>56</td>
<td>Right lower limb</td>
<td>100ml</td>
<td>3.15x10^7</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>No remarkable change Gait speed increased</td>
<td>Gait speed increased</td>
</tr>
</tbody>
</table>

### Discussion

The SVF derived from adipose tissue was originally described as mononuclear fraction and mitotically active source of adipocyte precursors by Hollenberg et al. in 1968 [8]. The SVF derived from adipose tissue is a heterogeneous cell population after enzymatic digestion of liposuction aspirate. The SVF contains large number of mesenchymal progenitor/stem cells, endothelial progenitors, preadipocytes, pericytes, T cells and M2 macrophages [9]. Mesenchymal progenitor/stem cells derived from SVF have capability to differentiate into diverse lineages of cells including adipocytes, osteocytes, and chondrocytes [10]. Despite the issues related to limited SVF yield, SVF demonstrates regenerative potential in the various damaged tissues through the paracrine mechanism [11]. Furthermore, SVF including T cell and M2 macrophage have potent immunoregulatory and anti-inflammatory abilities through the production of anti-inflammatory cytokines such as IL-10 [12]. Numerous applications of clinical implantation using autologous adipose tissue derived SVF in cosmetic surgeries support the safety and feasibility [13].

Clinical applications of SVF have been attempted to various conditions such as chronic wounds, osteoarthritis, Crohn’s disease, multiple sclerosis and other orthopaedic and neurologic conditions [14]. Their final results are not available yet. Though it’s still at investigative stage, SVF’s potential for clinical application would be very wide. However, quality control and safety issues of SVF extraction and isolation procedure are raised [15], and lack of regulatory guideline remains to be solved [16].

Since its introduction more than a decade ago, stem cell therapy raised expectation for potential benefit among subjects with poliomyelitis just as those with other kinds of disabilities. To our knowledge, this is the first report of stem cell therapy applied to individuals with poliomyelitis. Although there’s no established stem cell therapy for post-polio survivors yet, we assumed autologous adipose tissue derived SVF might be a clinically applicable stem cell therapy for this population.

Extraction of adipose tissue from abdominal subcutaneous fat layer and intramuscular injection of SVF was successfully performed without producing any significant adverse event in our participants. SVF cell isolation was satisfactory to yield sufficient cell counts with acceptable viability and colony forming...
units. The amount of progenitor cells in SVF cells was assessed by the number of colony forming unit. In this study, 10~60 million stromal cells per subject were isolated and roughly 2~15 million stromal cells were injected into a muscle. We could not find relationship between injected cell dose and clinical effect. There may be factors other than cell count that are related with clinical effect, such as genetic predisposition, exercise and behavioural factors, etc. We suppose our intervention procedure is safe and feasible in subjects with poliomyelitis.

The cases in this report suggest possibility of improving muscle function in poliomyelitis subjects by intramuscular injection of autologous adipose tissue derived mesenchymal stromal cells. The mechanism of increased muscle strength could be either local myogenic effect or systemic paracrine effect, or both. We call for further research to explore the myogenic potential of SVF so that it can be considered as one treatment option for those who have muscular weakness secondary to various kinds of neuromuscular diseases including post-polio syndrome.

Acknowledgement

This study was approved by the Institutional Review Board/Human Research Protection Office of International St. Mary’s hospital, the Catholic Kwandong University College of medicine (15IRB015, IS15OISE0016)

Disclosure of interest

The authors report no conflicts of interest

References