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Factors Affecting the Population of Mesenchymal Stem Cells in Adipose-Derived Stromal Vascular Fraction

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Background: Recently, the adipose tissue-derived stromal vascular fraction has become the most popular source for obtaining mesenchymal stem cells because it is less expensive and is easier to perform than bone marrow concentrate harvesting. However, no study has investigated the factors affecting the mesenchymal stem cell population in adipose tissue derived stromal vascular fraction. Understanding the interaction of patient factors with the mesenchymal stem cell count and cell viability in adipose tissue-derived stromal vascular fraction could provide crucial information for surgeons to improve patient selection and outcomes.

Aims: To evaluate the factors affecting the mesenchymal stem cell count, total cell count, and cell viability in adipose tissue-derived stromal vascular fraction.

Study Design: Retrospective cross-sectional study.

Methods: This study retrospectively reviewed the medical records of 30 patients who underwent liposuction to harvest adipose tissue-derived stromal vascular fraction at our stem cell center. Operative variables, such as lipoaspirate amount and donor areas from the stromal vascular fraction harvesting site, included the entire abdomen and lower abdomen. We recorded the mesenchymal stem cell population, cell viability, and cell count of stromal vascular fraction, and we analyzed the results to determine statistical significance.

Results: The factors that were found to be significantly related are as follows; between cell number and age (p=0.001) and amount of lipoaspirate (p<0.001); between cell viability and body mass index (p=0.005) and hypertension (p=0.047); and between coronary artery disease and mesenchymal stem cell counts (p = 0.028).

Conclusion: The relationship of patient factors (age, body mass index, hypertension, and coronary artery disease) with cell viability and mesenchymal stem cell counts may be important for clinical applications. However, the effect of medications on these relationships should be investigated in larger studies.

INTRODUCTION

Plastic surgeons introduced the use of adipose tissue (AT) as a grafting tool.[1] The stromal vascular fraction (SVF) consists of a multipotent stem and/or stromal cells.[2] The SVF is isolated from AT and has tremendous regenerative potential for proliferation and differentiation due to the so-called adipose-derived stem cells (ADSCs).[3] The mesenchymal stem cells (MSCs) are multipotent stem and/or stromal cells, and their ability to transform into mesodermic, endodermic, and exodermic cells gives them enormous potential in research and clinical applications in tissue repair.[4] They have specific lineages for their paracrine activity and interact with the microenvironment when transplanted into injured areas.

MSCs can induce new progenitors, produce various new cells, and facilitate the disposition of certain exosomes. The particles contain growth factors, cytokines, chemokines, and micro-RNA, thereby significantly restoring injuries and physiological functions.[5,6]

Secretion of these molecules enhances angiogenesis, immunomodulation, cell proliferation, and differentiation in injured tissues. ADSCs lack class II major histocompatibility complex sites, enabling them to serve in the allogenic setting without immune compromise.[7] However, emerging evidence suggests that the immunomodulatory effect of ADSCs is related to their regenerative ability.[8] The MSCs ameliorate pain, protect the tissue from infections, and, most importantly, prevent cytokine storms.[8,9]

Moreover, MSCs can repair tissues, including connective tissue and cartilage, by generating new structures. MSCs stimulate tissue and cartilage formation and inhibit inflammation.[10] This valuable attribute of MSCs has unlocked a new treatment era of arthrosis and

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related tissue injuries.[10] Implantation is effective, considering the migration capability of transplanted cells into target areas.[11]

MSCs can be isolated and harvested from various sources, such as the bone marrow, AT, peripheral blood, umbilical cord, synovial fluid, dental pulp, and endometrial tissues. However, not all sources provide clinically useful MSCs.[12] The bone marrow yields the most enriched and characterized MSCs for clinical use.[13] BMC was the most common procedure to prepare MSCs; however, bone marrow aspiration is highly invasive and results in low stem cell numbers.

Liposuction was extensively performed for patients worldwide, thereby increasing its popularity as an option for fat removal and body contouring. The main advantages of harvesting ADSCs rather than bone marrow are ease of access, safe and abundant harvesting, and low mortality risk. Unlike bone marrow, AT is very easy to collate and use for tissue engineering in large quantities without requiring an ex vivo expansion step.[14] Jang et al.[15] reported a higher MSC (CD45-CD31-CD90+CD105+) population in AT-derived stromal vascular function (AT-SVF; 4.28%) than in bone marrow sources (0.42%), and the adipose-derived stromal cell population (CD34+CD31-CD146-) was 32% in AT-SVF and 0.16% in bone marrow concentration (BMC). Recently, AT-SVF was studied as an alternate source of stem cells. AT-SVF contained approximately sixfold fewer nucleated cells than BMC; thus, adherent cells in AT-SVF were fourfold greater than BMC. Additionally, the colony-forming unit frequency of AT-SVF was higher than that of BMC at 0.5% and 0.01%, respectively.[15]

MSCs are identified by their adherence to plastic and expression of cell surface markers, including CD29, CD44, CD90, CD49a-f, CD51, CD73 (SH3), CD105 (SH2), CD106, CD166, and Stro-1 and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules based on the minimal criteria from the International Society of Cellular Therapy. MSCs have no immunogenic effect and could replace damaged tissue. These properties sparked the development of progressive methods to isolate and characterize MSCs from various sources for therapeutic applications in regenerative medicine.[16]

No previous study has investigated the factors affecting the MSC population in AT-SVF. These markers were individually analyzed in other studies, but we have investigated MSC-specific markers to assess the MSC population in AT-SVF.

The number of MSCs in the SVF is critical for determining the treatment effectiveness. The design phase of this research aimed to idntify the factors affecting the number of stem cells. We evaluated the MSC count (CD45-CD31-CD90+CD105+), total cell count, and factors affecting total cell viability. Analytical planning of the clinical use of the SVF also plays an essential role in planning effective patient treatment. Additionally, we reviewed the effectiveness of demographic factors and clinical and surgical data on the obtained cells and cell viability in the SVF.

MATERIALS AND METHODS

This study retrospectively reviewed the chart of 30 patients who underwent liposuction to harvest adipose-derived SVF at our stem cell center from September 2018 to August 2019. We analyzed electronic medical records for demographic, clinical, operative characteristics, and outcomes. Patient demographic and clinical data (age; gender; body mass index [BMI]; blood type; medical comorbidities, such as diabetes, hypertension, and coronary artery disease [CAD]; smoking; and alcohol use) were noted from the hospital database. We recorded the amount of lipoaspirate and the donor areas from where the SVF was harvested, including the entire abdomen and lower abdomen, for operative variables. The viability and cell count of SVF were documented.

Fat Harvesting

All operations were performed under sedoanalgesia in a sterile operating room. We used the tumescent technique and harvested lipoaspirates from the abdominal region. The same plastic surgeon performed all operations.

Umbilical and suprapubic areas were selected as entry sites. The tumescent solution, containing ringer lactate with 1:500,000 units of epinephrine and 20 mg of bupivacaine hydrochloride, was infiltrated through the 3-mm incisions and allowed to rest for 10 min to achieve hemostasis. The volume of tumescent solution injected was 150 cc.

Following the hemostasis, a blunt cannula (the "Fat Grater") with a 2-mm diameter, of which the tip has 10 holes measuring 1.5 mm in diameter, was utilized to harvest the adipose aspirate. The attached cannula to a 60-ml syringe and the gentle pulling back on the syringe's plunger provided a light negative pressure while the cannula was advanced and retracted through the harvest site. The cannula was removed from the syringe after filling the syringe with harvested tissue, and the lipoaspirate was transferred into sterile 50-ml Falcon tubes for further enzymatic processing.

Isolation of the SVF

All production processes were conducted following Good Manufacturing Practices. Fat tissue samples taken from the patient were placed in Falcon 50-ml Polypropylene Conical Tube (Avenida Industrial del Norte S/N, Parque Industry del Norte, Reynosa, Tamaulipas, Mexico). A sample was drawn from the tissue with an injector, and an input sample was given for microbiological quality control.

The AT samples in the tubes were arranged in Falcon tubes, and the collagenase enzyme (SIGMA-ALDRICH, St. Louis, MO, USA) was placed in each Falcon tube. The collagenase enzyme had been sterility controlled, and the endotoxin level was approved as <10 IU/ml before use. The caps of the tubes were tightly closed, and the tubes were placed in the shaker. The shaker was adjusted to 100 rpm, and the tubes were incubated for 60-90 min.

After completing the incubation, the tubes were directly placed in the centrifuge device and centrifuged at 500xg for 10 min. After centrifugation, the supernatant was removed using a pipette, and the pellet containing the cells was manually vortexed.

The pellet was removed and collected in a single tube via pipette. Filtering was done into a new tube with Cell Strainer, and the

volume was completed with Ringer Lactate Solution. The mix was spun again in a centrifuge for 10 min at 500xg.

Following the second round of spinning, the supernatant was removed, Ringer Lactate Solution was added to the pellet in the desired amount, and the mixture was centrifuged for a third time at 500xg for 10 min.

Quality Control Tests

The sample was drawn from the supernatant with an injector and run through the BD BACTEC Automated Blood Culture System (BD, East Rutherford, NJ, USA) for aerobic and anaerobic cultures and the fungus culture sample was obtained to assure microbiological quality control after the final centrifugation. Additionally, we analyzed endotoxin levels. The product was released for microbiological culture results once the Gram staining was negative in the sampling. The culture results were monitored for 2 weeks.

SVF Counting, Viability Assessment, and Immunophenotyping

Red blood cells were lysed via lysis buffer (eBioscience, CAT No: 000-433357). We used a hemocytometer to perform the cell count. We assessed cell viability using flow cytometry analysis for 7-aminoactinomycin D staining.

Immunophenotyping was performed on SVF for flow cytometry analysis. Cell surface antigen expression of AT-derived MSCs was detected using flow cytometry. We evaluated positive versus negative expression by flow cytometry using CD45, CD34, CD105, and CD90 monoclonal antibodies, with Cell Quest software (BD, East Rutherford, NJ, USA). Then, we calculated which cells were negative for CD45 and CD34 and which were positive for CD90 and CD105 as MSCs, and the percentage in the total population was calculated.

Statistical Analysis

Patient data collected within the scope of the study were analyzed with IBM Statistical Package for the Social Sciences for Windows, Version 23.0. (IBM Corp., Armonk, NY). Moreover, the power analysis was performed using Power Analysis and Sample Size Software version 11.0. (PASS, NCSS Statistical Software, Kaysville, UT; Table 1). The power analysis revealed an 81.6% power of the study. Frequency and percentage were given for categorical data and median, minimum, and maximum descriptive values for continuous data. The Shapiro-Wilk test examined the conformity of continuous quantitative variables to Gaussian distribution. The Mann-Whitney U test was used to compare two groups and the Kruskal-Wallis H test for three groups. The relationship between continuous variables was evaluated using Spearman's Correlation test. The results were considered statistically significant with *p*-values of <0.05.

RESULTS

This retrospective analysis included 30 patients. The median age was 57 years (range, 25-85 years). Most of the study population was female (76.7%, n=23), and 23.3% (n=7) were male. The median BMI of the study population was 30 kg/m^2 (range, 18.6-51.9 kg/m^2). Blood type A+ was the most common (56.7%, n=17), followed by O+ (26.7%, n=8), B+ (10%, n=3), AB+ (3.3%, n=1), and O- (3.3%, n=1). Hypertension is the most common comorbidity (40%, n=12), followed by CAD (30%, n=9), smoking (20%, n=6), diabetes (16.7%, n=5), and alcohol consumption (3.3%, n=1; Table 2).

Table 3 presents the correlation analysis to determine the nature of the relationship between MSC count (CD45 and CD34-/CD90 and CD105+), cell count, cell viability, and demographic and clinical findings. No statistically significant relationship was found between MSC count and other parameters ($p>0.05$; Table 3).

The median cell count was 45x10^6, and the cell viability was 94 (range, 86-100). The lipoaspirate amount was 140 (range, 50-212). MSCs had a mean incidence of 2.25% (range, 0.25%-12.45%) in AT-SVF (Table 2).

We determined a positive linear correlation between cell count and age, and lipoaspirate amount ($p<0.05$) but found no statistically significant relationship with other variables ($p>0.05$). We noted a positive linear relationship between cell viability and BMI ($p<0.05$) but no other variable (Table 3).

The Mann-Whitney U and Kruskal-Wallis H tests were performed to evaluate the significant difference in demographic, clinical, and operative findings in cell count, cell viability, and MSC numbers are shown in Table 4. Hypertension was significantly associated with cell viability, and CAD was significantly associated with MSC count ($p<0.05$). Cell viability in patients with hypertension was higher than in those without hypertension, and the MSC count of individuals with CAD was higher than in those without CAD. No statistically significant difference was found in the number of cells, cell viability, and MSC (CD45 and CD34-/CD90 and CD105+) count among other variables ($p>0.05$).

TABLE 1. Normality Test Results for Variables

Variables (n=30)	Statistics	Degrees of freedom	*p*-value
Lipoaspirate amount	0.180	30	0.020
Cell count	0.178	30	0.015
CD45 vs. CD34 (−)/CD90 vs. CD105 (+)	0.227	30	<0.001
MSC (viability)	0.218	30	0.001

MSC: Mesenchymal stem cell

TABLE 2. Distribution of Demographic, Clinical, and Operative Findings of Participants

Characteristics (n=30)	n (%)	Median (range)
Gender		
Female	23 (76.7)	
Male	7 (23.3)	
Age in years		57 (25-5)
BMI in kg/m^2		30 (18.6-51.9)
Blood type		
0+	8 (26.7)	
0-	1 (3.3)	
A+	17 (56.7)	
B+	3 (10.0)	
AB+	1 (3.3)	
Diabetes	5 (16.7)	
Hypertension	12 (40.0)	
Coronary arctery disease	9 (30.0)	
Smoking	6 (20.0)	
Alcohol consumption	1 (3.3)	
Donor zone		
Lower abdomen	29 (96.7)	
Entire abdomen	1 (3.3)	
Lipoaspirate amount		140 (50-212)
Cell count		45x10^6 (14x10^6 to 84x10^6)
Mesenchymal stem cell count		
(CD45(−)CD34(−)/CD90(+)CD105 (+))		2.25% (0.25%-12.45%)
Cell viability		94% (86%-100%)

BMI: Body mass index

TABLE 3. Evaluation of Viability, Cell Count, and Mesenchymal Stem Cell Counts According to Demographic, Clinical, and Operative Findings

Spearman's correlation		Age	BMI	Lipoaspirate amount	Cell count	Viability	Mesenchymal stem cell count
Mesenchymal stem cell count	Correlation coefficient	-0.048	0.355	-0.047	-0.286	0.042	1.000
	p-value	0.802	0.054	0.804	0.125	0.827	-
Cell count	Correlation coefficient	**0.576**	0.212	**0.610**	1.000	0.137	-0.286
	p-value	**0.001**	0.260	**<0.001**	-	0.472	0.125
Cell viability	Correlation coefficient	0.224	**0.499**	0.041	0.137	1.000	0.042
	p-value	0.233	**0.005**	0.831	0.472	-	0.827

BMI: Body mass index

DISCUSSION

This study represents the first study conducted on the correlation of demographic, clinical, and surgical data on the obtained MSCs and cell viability in the AT-SVF. We found a positive correlation between MSC count and CAD, which was not previously reported in the literature. Previous studies have focused on MSC-based therapy for cardiovascular diseases rather than cell count. In another milestone discovery, this study found a positive correlation between cell viability and hypertension. However, the positive correlation could be due to increased intraluminal pressure, which might have facilitated cell viability. Additionally, calcium-channel blockers might deteriorate cellular attachment, thereby increasing harvested cells.[17] Previous articles on MSCs and hypertension elaborated on the ameliorating effects of MSCs on hypertension-related pathologies and improvement in renovascular hypertension.[18-20]

Age is a strong determinant of ADSC quality because younger patients hold greater proliferative capacity and are more efficient at differentiating into mature adipocytes than older patients.[21]

TABLE 4. Distribution of Characteristics by Cell Count, Cell Viability, and Mesenchymal Stem Cell Count Measurements

Characteristics (n=30)	Median cell count, x 106/µl (range)	*p*-value	Cell viability median (range)	*p*-value	Mesenchymal stem cell count median (range)	*p*-value
Gender		0.961		0.692		0.135
Female	44 (20-84)		94 (86-100)		2.15 (0.25-12.45)	
Male	49 (14-76)		97 (90-98)		3.55 (0.45-6.80)	
Blood type		0.173		0.406		0.508
O+	59 (40-73)		97 (90-98)		1.88 (0.25-6.30)	
O-	44 (44-44)		98 (98-98)		2.15 (2.15-2.15)	
A+	41 (14-76)		92 (86-100)		2.50 (0.55-12.45)	
B+	41 (27-84)		91 (90-97)		2.25 (1.55-6.80)	
AB+	35 (35-35)		93 (93-97)		1.75 (1.75-1.75)	
Diabetes		0.781		0.933		0.182
No	44 (14-84)		94 (86-100)		2.20 (0.25-12.45)	
Yes	48 (40-54)		97 (89-97)		2.50 (1.80-7.25)	
Hypertension		0.107		**0.047**		0.512
No	40.5 (14-84)		**92.5 (86-98)**		2.17 (0.25-7.25)	
Yes	51.5 (27-76)		**97 (90-100)**		2.30 (0.45-12.45)	
Coronary artery disease		0.683		0.398		**0.028**
No	46 (14-84)		94 (86-99)		**2.00 (0.25-7.25)**	
Yes	41 (27-76)		97 (90-100)		**3.20 (1.75-12.45)**	
Smoking		0.876		0.173		0.795
No	45 (14-76)		94.5 (89-100)		2.30 (0.25-12.45)	
Yes	45 (20-84)		91.5 (86-97)		2.23 (1.45-7.25)	
Alcohol consumption		0.133		0.091		0.817
No	46 (14-84)		94 (89-100)		2.25 (0.25-12.45)	
Yes	20 (20-20)		86 (86-86)		2.35 (2.35-2.35)	
Donor zone		0.916		0.275		0.409
Lower abdomen	42.5 (20-84)		96 (89-100)		2.08 (1.20-12.45)	
Entire abdomen	49 (14-76)		93 (86-98)		2.58 (0.25-7.25)	

Therefore, younger patients are more suitable for tissue engineering applications than older patients. However, our results show a positive linear correlation between cell count and age which we did not expect and seems to contradict conventional knowledge. This relationship needs further exploration with larger populations and additional research to reconcile this trend against the current literature.

Andjelkov et al.[22] assessed the correlation of age, gender, BMI, and smoking status with the SVF cell yield and viability, and they indicated that age significantly influenced SVF cell yield positively, as we found in our study. BMI and gender did not influence the cell yield or the SVF viability. Smoking was significantly negatively associated with SVF cell yield. Contrary to our results, smoking did not influence cell viability or yield in SVF. Our study revealed no significance between the MSC count and smoking or alcohol consumption. To our knowledge, no previous study has assessed such relationships. Further studies with larger sample sizes revealed that smoking and alcohol consumption affected the MSC count in AT-SVF. Additionally, we found that blood type did not influence MSC count, cell viability, or cell yield in SVF-an association that had not been explored previously in the literature.

Moreover, our study revealed higher cell count in patients with higher lipoaspirate amounts ($p<0.05$). ADSCs' proliferation capacity and functionality are heavily influenced by the patient's BMI, with increased activity in some massively obese patients than in lean controls.[23] Our study revealed a positive linear relationship between cell viability and BMI ($p<0.05$), which might be due to the high BMI of the individuals. The median BMI of our study population was 30 kg/m^2.

This study evaluated the specific surface markers of MSC in AT-SVF. A previous research individually investigated cell surface markers[20,21,23,24] and checked only for CD90 expression which has

limited implications. MSC should be distinguished from other cells in SVF by identifying the expression of specific antigens, such as CD34 and CD45-negative and CD90 and CD105-positive, to assess the content of MSC in AT-SVF.

Our study performed enzymatic dissection to harvest MSC instead of mechanical dissection due to the greater recovery associated with enzymatic dissection than mechanic dissection. Additionally, enzymatic dissection brings cells into a single-cell suspension. However, cell clusters usually remain after mechanical dissection, causing uneven applications at the injection sites. Further, cell clusters cause microcalcification and necrosis in the late period. The only disadvantage of enzymatic dissection is the high cost associated with Good Manufacturing Process (GMP). We used the Liberase MTF C/T, GMP Grade kit containing highly purified collagenase class I and class II from *Clostridium histolyticum*.

AT is known for its energy storage capacity. Additionally, AT contains a high number of stromal cells. AT-SVF provides 500 times more stem cells than bone marrow.[25] van Harmelen et al.[25] reported a constant ratio of adipocytes to MSC in humans, independent of BMI and age. Therefore, patients with a high BMI would have a larger total MSC harvest than patients with a low BMI. Similarly, we observed a positive linear relationship between cell viability and BMI.

Chen et al.[1] reported >95% positivity of CD44, CD29, and CD59 MSCs. They have also stated that negative suction pressure did not influence the expression of the isolated ADSC surface markers. They investigated ADSC surface marker expression parameters rather than MSC count. However, our study identified MSCs by detecting CD45 and CD34-/CD90 and CD105+ surface markers in the SVF.

Another critical issue regarding the effect of centrifugal force on adipocytes is their sensitivity to mechanical forces, including pressure. Our study revealed a 500 RPM centrifuge speed for 10 min which was tested and validated for the cell viability using the trypan blue staining and function tests (with proliferation index). Acıbadem Labcell is a laboratory with cGMP accreditation that conducted the validation of this speed.

Debnath and Chelluri[26] reported no effects of the donor site on SVF cell count. The SVF ratios did not differ for CD31, CD34, CD44, CD90, or CD45 expression. SVF expressed the stem cell-associated marker of CD44 at 74.2%, CD34 at 62.9%, CD90 at 55.1%, and CD31 at 19.3%.[26] We found that donor sites did not affect MSCs count, cell count, or viability.

Our study design was limited by the lack of a randomized protocol and the small patient population. Additionally, the drugs or medicines used by patients likely affected several conditions, but we did not examine those interactions because this research was not designed to measure drug-related parameters. Despite these limitations, our study is the first to examine the factors affecting the MSC count in the SVF in a detailed manner.

This was the first study conducted on the effects of patient demographic, clinical, and surgical data on the MSC count and cell viability harvested from AT-SVF. We found three positive linear correlations; first, between MSCs count and CAD; second, between cell count, age, and lipoaspirate amount; and third, between cell viability, hypertension, and patient BMI. These correlations may have potential clinical applications. However, the impact of medications on these relationships must be explored in future studies with larger sample sizes to further elucidate these associations.

Ethics Committee Approval: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of our institution (2021-20/31; 10/2021).

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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