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Impact of Age, Body Mass Index, and Harvesting Sites on the Regenerative Properties of the Adipose Derived Stem Cells in Egyptians

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ABSTRACT

Adipose tissue is proved to be most viable source of the stem cells. The choice of the site of harvest of the adipose tissue for stem cells isolation and the patient's age and body mass index is addressed to evaluate their relation to the population and viability of stem cells.

In this study we operated upon 84 patients. From each patient fat tissue was harvested by manual liposuction from 3 sites (abdomen, medial thigh and back), then the adipose derived stromal cells were enzymatically isolated. Assessment of its population and viability was done the trypan blue exclusion test.Results were statistically analyzed according to their age body mass index and harvesting sites.

The average cell yield was 0.389 x 10^6/ml (average number of abdominal samples was 0.380 x 10^6, medial thigh samples 0.352 x 10^6 and back samples 0.260 x 10^6). Viability of adipose derived stromal cells from different sites, ages and body mass index range was (96-100%). The results from this study suggest that there is statistically highly significant negative correlation between patients' age and body mass index and adipose derived stromal cells population with no suchcorrelation regarding viability. So, the choice of the donor site for adipose derived stromal cells should be based on ease and safety of access and patient preference.

Key Words: *Stem cells – Harvesting sites – Body mass index – Age – Regenerative properties.*

INTRODUCTION

The regenerative properties of the mesenchymal stromal cells have been recognized many years ago. Isolation of them was reported from many tissues such as; amniotic fluid, umbilical cord blood, synovial fluid, bone marrow, peripheral blood, and the adipose tissue. The choice of the site of isolation of them depends on many factors for example; the ease of harvest and their population **[1-7].**

Adipose tissue was defined as a new source of mesenchymal stromal cells. The adipose tissue contains stromal vascular fraction that contain population of adipose tissue derived stem cells (ACSc) **[8].**

Using the adipose derived stem cells has many advantages. One of them is the large number of the stem cells that can be isolated from adipose tissue in comparison to other sources, for example bone marrow. In addition to the ease of harvest of the adipose tissue, which carry minimal risk to the patients with slight or no discomfort **[9].**

Previously, the adiposed tissue derived stem cells was prepared enzymatically from lipoaspirate. As it was found in the stromal vascular fraction cell population **[8].** Currently, the perivascular tissues are the main site of the adipose derived stem cells concentration **[10].**

Our aim in the current study is to address whether the age, body mass index or site of harvest significantly affect the population and viability of adipose derived stem cells in Egyptian people.

PATIENTS AND METHODS

This prospective study was conducted during the period from July 2014-January 2016 on 84 patients presenting for liposuction +/- lipotransfer. Inclusion criteria comprised healthy patients with age from 18 to 65 years, body mass index (BMI) from 18.5 to 35kg/m^2 according to WHO classification of BMI.

Exclusion criteria were patients with age below 18 and above 65 years, patients of body mass index below 18.5, patients of nationality other than Egyptians, patients class III till class VI according to American Society of Anesthesiologists (ASA) Physical Status classification system. Fat tissue

was harvested during elective body contouring (liposuction and/or lipotransfer) procedures from 3 different sites (abdomen, medial thigh & back).

Adipose tissue harvesting:

The procedure was performed under local anesthesia, (Tumescent local anesthesia, TLA). The fat tissue aspirated from using manual aspiration into a syringe using 3mm multi holes cannula. Immediately following collection, the tissue collection vessel will be transported to the laboratory at ambient temperature.

Isolation of ASCs and assessment of yield and viability:

SVF will be isolated from collagenase enzyme-digested lipoaspirate using the following steps: Each 25cc lipoaspirate washed several times in equal volumes of phosphate buffer saline till the specimen becomes clear. Clarify the specimen from the excess fluid. Digestion of fat will then be undergone using equal amount of collagenase enzyme solution. Placing the mixture in culture flask and put in a shaking water bath at 37ºC for 1 hour. The digested fat will be transferred to a conical tube and washed in Dulbecco's Modified Eagle's medium (DMEM) buffer solution. Lastly centrifuge at 4000 rpm for 5 minutes to obtain a pellet. The pellet is then resuspended in a buffer solution and suspended cells are counted using a hemocytometer. Cell viability is calculated by adding one drop of trypan blue to one drop of cell suspension and the number of nonviable cells taking up the blue stain is counted and the percentage of viable cells is deduced.

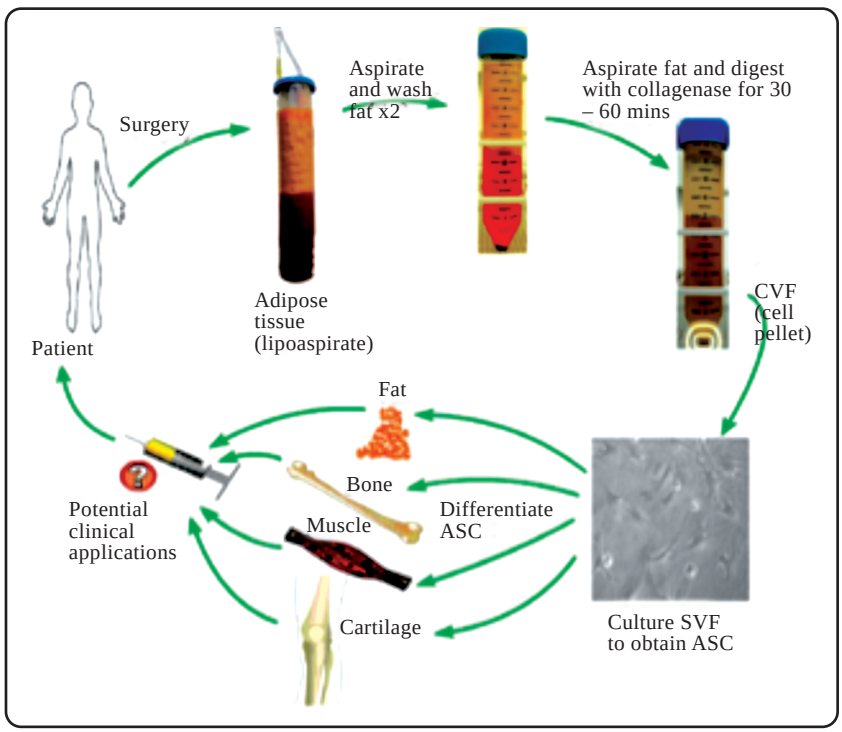

Fig. (1): Summary of cycle of human adipose-derived stem cells isolation and differentiation for clinical usage **[11]**.

Statistical methodology:

Descriptionof quantitative variables as mean, SD and range was done by using the SPSS software (statistical program for social science version 12).

The one-way analysis of variance (ANOVA) is used to determine whether there are any significant differences between the means of three groups regarding the viability of stromal cells.

The Kruskal-Wallis H test was used to determine if there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable. It allows the comparison of more than two independent groups.

RESULTS

The total number of cases was 84 patients. They were divided according to their age into three groups and according the BMI they were divided into three groups. The fat was harvested from three sites in each patient. The stromal cells yield and

viability according to the harvesting sites is demonstrated in the following two tables.

The patients were divided into three groups according to the age, the following two table shows comparison between the stromal cell yield and viability according to patient's age.

The following two tables summarize the relation between the stromal vascular population and viability according to BMI.

There was highly significant negative statistical correlation found between the age and population of stromal cells in different harvested samples. There is also highly significant negative correlation between the BMI and the stromal cells population (Table 7).

The following table shows that there is statistically non-significant correlation between either age or BMI and the stromal cells viability.

The following table shows that there was highly statistically significant positive correlation found between the three harvesting sites each other as regarding the population of stromal cells.

The following table shows that there was highly statistically significant positive correlation found between the three harvesting sites each other as regarding the viability of stromal cells.

Table (1): Comparison between the three harvesting sites regarding yield of stromal.

	Median (IQR)	Kruskall-Wallis test	
		K	*p*-value
Abdominal sample (x10^6)	18.8 (9-32)		0.064 NS
Thigh sample (x10^6)	17.6 (5.4-29.5)		
Back sample (x10^6)	13 (3.3-24.4)		

IQR: Interquartile range.
p-value • 0.05 (NS) non-significant.
p-value • 0.05 (S) significant.
p-value • 0.01 (HS) highly significant.

Table (2): Comparison between viability of stromal cells in different harvesting sites.

	Range	Mean ± SD	One way ANOVA	
			F	*p*-value
Abdominal sample viability (%)	95-100	97.74±1.32		
Thigh sample viability (%)	96-100	97.79±1.26	0.872	0.420 NS
Back sample viability (%)	96-100	98.06±1.33		

Table (3): Comparison between yield of stromal cells in different age groups.

Stromal cells population	Group 1 18-37 years	Group 2 38-45 years	Group 3 46-65 years	Kruskall-Wallis test	
	Median (IQR)	Median (IQR)	Median (IQR)	K	*p*-value
Abdominal Samples (x10^6)	33 (24-45)	20 (15-27)	6 (2-9)	52.151	0.000 HS
Thigh Samples (x10^6)	32 (20.5-36.8)	20 (13.4-25.5)	3.3 (2.9-6.5)	50.847	0.000 HS
Back Samples (x10^6)	24.8 (13.1-33.8)	17.9 (12.5-23)	2.3 (1.7-3.3)	53.226	0.000 HS

Table (4): Comparison between viability of stromal cells in different age group.

	18-37 Years	38-45 Years	46-65 Years	One way ANOVA	
	No.=28	No.=28	No.=28	F	*p*-value
Abdominal sample viability (%):					
Mean±SD	97.48±1.24	98.19±1.23	97.85±1.37	2.189	0.119 NS
Range	95-99.5	96-100	96-100		
Thigh sample viability (%):					
Mean±SD	97.55±1.18	98.02±1.23	97.80±1.37	0.982	0.379 NS
Range	96-99.5	96-100	96-100		
Back sample viability (%):					
Mean±SD	97.79±1.25	98.45±1.14	98.16±1.44	1.869	0.161 NS
Range	96-100	96.5-100	96-100		

Table (5): Comparison between yield of stromal cells in different BMI groups.

	BMI 20-24.9	BMI 25-29.9	BMI >30	Kruskall-Wallis test	
	Median (IQR)	Median (IQR)	Median (IQR)	K	*p*-value
Abdominal Samples (x10^6)	28 (19-39)	22 (13-32)	9 (5-17)	24.690	0.000 HS
Thigh Samples (x10^6)	32.1 (16-38)	20.5 (11.9-30.5)	5.5 (3.3-13.4)	25.139	0.000 HS
Back Samples (x10^6)	24.8 (13-33.8)	15.3 (11.7-23.4)	3.2 (2-12.5)	21.880	0.000 HS

Table (6): Comparison between viability of stromal cells in different BMI groups.

	BMI 20-24.9	BMI 25-29.9	BMI >30	One way ANOVA	
	No.=22	No.=32	No.=30	F	*p*-value
Abdominal sample viability (%):					
Mean±SD	97.82±1.13	97.78±1.37	97.92±1.37	0.082	0.921 NS
Range	96-99.5	95-100	96-100		
Thigh sample viability (%):					
Mean±SD	97.86±1.17	98.03±1.26	97.49±1.31	1.458	0.239 NS
Range	96-99.5	96-100	96-99.5		
Back sample viability (%):					
Mean±SD	97.70±1.23	98.32±1.26	98.26±1.34	1.762	0.178 NS
Range	96-100	96-100	96-100		

Table (7): Spearman correlation between (age, BMI) and studied samples regarding stromal cells population.

	Age (year)		BMI	
	r	*p*-value	*r*	*p*-value
Abdominal sample (x10^6)	–0.790**	0.000 HS	–0.503**	0.000 HS
Thigh sample (x10^6)	–0.779**	0.000 HS	–0.509**	0.000 HS
Back sample (x10^6)	–0.785**	0.000 HS	–0.472**	0.000 HS

Table (8): Spearman correlation between (age, BMI) and studied samples regarding stromal cells population.

	Age (year)		BMI	
	r	*p*-value	*r*	*p*-value
Abdominal sample viability %	0.105	0.343 NS	–0.013	0.908 NS
Thigh sample viability %	0.092	0.407 NS	–0.120	0.277 NS
Back sample viability %	0.099	0.369 NS	0.135	0.222 NS

Table (9): Spearman correlation between the three harvesting sites regarding stromal cells population.

	Abdominal sample (x10^6)		Thigh sample (x10^6)		Back sample (x10^6)	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
Abdominal sample (x10^6)			0.781	0.000 HS	0.756	0.000 HS
Thigh sample (x10^6)	0.781	0.000 HS			0.866	0.000 HS
Back sample (x10^6)	0.756	0.000 HS	0.866	0.000 HS		

Table (10): Spearman correlation between the three harvesting sites regarding stromal cells viability.

	Abdominal sample viability (%)		Thigh sample viability (%)		Back sample viability (%)	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
Abdominal sample viability (%)			.598	0.000 HS	.678	0.000 HS
Thigh sample viability (%)	.598	0.000 HS			.509	0.000 HS
Back sample viability (%)	.678	0.000 HS	.509	0.000 HS		

DISCUSSION

Using the autologous fat transfer in management of soft tissue volume loss and volume augmentation has been described many years ago. It is attractive solution because of ease of harvest; low risk of the procedure as well as large volume can be harvested.

The factors that affect the viability of the fat grafting were analyzed by many researches. All of these studies were concerned with the mature adipocytes **[12-15].**

Hence, the adipose derived stem cells (ASCs) are supposed to be a favorable precursor for use in regenerative medicine, as it can be enzymatically isolated and concentrated. The distribution of the ASCs in human adipose tissues as well as the factors affect the viability of them are not clarified yet. Therefore, it is important to address the distribution of ASCs to obtain cell populations rich in viable ASCs for clinical purpose.

The previous reports that address the impact of the harvesting site on the fat cell number and viability for example Rohrich et al. **[16]**; their conclusion was that the site of harvest doesn't affect either the population or the viability of the fat cells after comparing the abdomen, flank, thigh, and medial knee regions.

Von Heimburg et al. **[17]** addressed the effect of the site and method of harvest on the preadipocytes. They conclude that there is comparable viability regardless the site of harvest (abdomen, breast, or buttock) or method of harvest (either the excisional or liposuction). This confirms our results as we used abdominal region, inner thigh and back region as donor sites in each case in this study. The results showed no statistically significant differences between the three harvesting sites regarding stromal cells populationor viability.

In other reports such as Faustini **[18]**, it was shown that ASCs yield from the abdominal region in males is more significant. Paoin et al. **[19]** showed that the abdomen and inner thigh yield higher lipoaspirate cell population in comparison to other regions.

The conclusion from these data seems likely that donor site choice plays a minimal role in the yield and viability of ASCs, and choosing a site should be based on ease and safety of access and patient preference.

As regard the impact of BMI on theASC cell yield, our study indicates that the yield of ASCs might be influenced by the BMI of the donor as we found a highly statistically significant negative correlation. Also Aust et al., **[20]** reported a negative correlation between ASC concentration and BMI. A similar significant negative correlation between cell yield and BMI was shown by Van Harmelen et al. **[21]**.

Yu G **[22]** reports a positive correlation of ASC yield and BMI. Other studies determined no significant influence of BMI on cell yield. For example, Buschmann, et al. **[23]**, Mojallal A., et al. **[24]** and Yoshimura, et al. **[25]**.

Effect of age on the proliferation capacity of mesenchymal stromal cells in both human and mouse have been studied **[26]**. However the effect of age on the ASCs has been partially studied. Reports dealing with the influence of age, on yields and proliferation rates vary greatly in their outcomes. For example, Yu, et al. **[22]** claim to have found a positive correlation between cell yield and donor age. Buschmann **[23]** study also found a significantly higher ASC cell yield of donors aged 38-44 years compared with older donor's ages >45 years. In contrast Faustini M., et al. **[18]** report higher cellular ASC yields for female donors >45 years of age compared with female donors <35 years of age. Girolamo LD et al. **[27]**, Showed a significant positive correlation between age and cell yield. Cell viability and in vitro adipocytic differentiation showed no significant difference between the studied groups (<35 years and >45 years). Nevertheless, younger donors (20 year olds) showed a twofold increase, which was, however, statistically insignificant.

Our study indicate that the yield of ASCs might be influenced by the age of the donor because we found a significantly higher ASC cell yield of donors aged 18-37 than donors aged 38-44 than those with older donors ages >45 years (highly significant negative correlation). Our results showed highly significant negative correlation regarding ASCs population and no correlation regarding ASCs viability.

We were confronted by many limitations during the study. One of them is only one method was used to quantify cell yield: The cell counting and cell viability enzymatic digestion with trypan blue exclusion test. Trypan blue exclusion test depends on the membrane stability to assess the cell viability, it is a standardized and accepted test for monitoring cell viability. However, the trypan blue exclusion test should be coupled with the growth kinetics

assay, to create a clear picture of the yield of viable ASCs.

Other limitation of this study may be that there were only 3 patients above 55 years included only one above 60 years who sought body contouring procedures, so that we couldn't assess the effect of older ages on ASCs yield.

Also body contouring procedures are rarely to be done to patients with BMI >40. So there were only 4 patients with BMI >40 in this study, and hence we couldn't recruit more patients to detect the impact of morbid obesity on yield of ASCs.

Another limitation may be that we get fat to be examined from only 3 harvesting sites as procedures were performed under local anesthesia and we chose these sites due to ease & safety of access and patients preference; however other areas need to be studied for their yield of ASCs.

In the future we suggest following-up the patients who undergo fat transfer to assess if there is any relationship between the survival & resorption rates of the fat grafts, and the high or low yield of ASCs from different ages, BMI and harvesting sites.

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