Yield of Adipose-derived Stromal Cells as the Influence of Body Mass Index in Middle Age Group of Egyptian Patients

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ABSTRACT

Adipose-derived stem cells (ADSCs) considered to be a subtype of the mesenchymal stem cells (MSCs). They have the same regenerative capabilities like other MSCs. However, the abundance of ADSCs and the minimal morbidity and accessibility of their harvest and preparation have led to a booming the research work on it. The plastic surgeons are inimitably positioned to utilize this technology as they are frequently perform liposuction and fat transfer procedures in their everyday practice. The primary purpose of this research is to determine the influence of BMI index over the population and viability of adipose derived stem cells (ADSCs) in Egyptian patients of the same sex, age group and from same fat harvesting site. In this study we operated upon 63 patients. From each patient fat tissue was harvested by manual liposuction from abdomen, and 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 body mass index. The average cell yield was 0.380 x 10^6 ml. Viability of adipose derived stromal cells from different body mass index range was (96-100%). The results from our study advocate that there is statistically highly significant negative correlation among patients' body mass index and adipose derived stromal cells population with no such correlation regarding viability.

Key words: *Adipose-derived stem cells, Body mass index, population and viability of ADSC, middle age population.*

INTRODUCTION

The current researches of different types and sources of mesenchymal stem cells (MSCs) have great impact in expanding the field of regenerative medicine. MSCs considered as non-hematopoietic cells and can be harvested from different organs and connective tissues [(1)]

It have been harvested and prepared from different tissue sources including periosteum [(2)], adult trabecular bone [(3)] , periodontal ligament [(4)], skeletal muscle [(5)], skin [(6)], pericytes [(7)], deciduous teeth [(8)], peripheral blood [(9)], synovial membrane [(10)] and umbilical cord [(11, 12)].

Unfortunately, the quantity of stem cells population derived from the previously mentioned source are usually inadequate to be used clinically in regenerative medicine. As well as the other encountered problem is due to paucity of these tissue as a donor sites , the adult derived stem cells need manipulation before being used. The ex vivo expansion became a mandatory step.

Thinking of other easily accessible as well as rich source of stem cells became essential to permit continuation of more advanced studies in field of tissue regeneration. So, the adipose-derived stem cells (ADSCs) [(13)], are one of the utmost promising stem cell population accepted thus far. The adipose tissue is abundant and can be easily harvested in suitable quantity with no fear of donor site morbidity or patient embarrassment [(14)]. Consequently, the usage of autologous ADSCs as research tool and cellular therapeutics is feasible and has been shown to be both harmless and efficient in both clinical and preclinical studies [(15)].

As the field of plastic surgery, focused on the recontouring and reconstruction of the body, is logically positioned to utilize such technologies focused on the repair and replacement of diseased cells and tissues [(16)].

The primary purpose of this study is to determine the influence of BMI index over the population and viability of adipose derived stem cells (ADSCs) in Egyptian female patients of the same age group and from same fat harvesting site.

PATIENTS AND METHODS

This prospective study was conducted during the period from July 2014-January 2017 on 63 patients presenting for liposuction ± lipotransfer. Inclusion criteria comprised healthy female patients with age from 31 to 50 years (middle age group according to Anuradha Yarlagadda.) (17), body mass index (BMI) from 18.5 to 35 kg/m2 according to WHO classification of BMI.

Exclusion criteria were male patients, patients with age below 31 and above 50 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 abdominal region in all included cases.

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 3 mm multi holes cannula. Instantly following collection, the tissue collection vessel will be transferred to the laboratory at ambient temperature.

Isolation of ASCs and assessment of yield and viability:

Stromal vascular fraction (SVF) will be isolated from collagenase enzyme-digested lipoaspirate using the following steps: Each 25 cc 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 37^0C 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.

Statistical Methodology:

Description of quantitative variables as mean, range and Standard Deviation (SD) and 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 the presence of any significant differences between the means of three groups regarding the viability of stromal cells.

The Kruskal-Wallis H test was used to determine the presence of 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 63 female Egyptian patients. They were divided according to their BMI into three groups each group 21 patients. The fat was harvested from the abdominal region in each patient. The stromal cells yield and viability according to ***BMI index*** is demonstrated in the following two tables.

Table (1): 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 (× 10^6)	26(18 – 38)	23(14– 32)	7(3 – 16)	25.580	0.000 HS

Table (2): Comparison between viability of stromal cells in different BMI groups

		BMI 20 - 24.9	**BMI 25 - 29.9**	**BMI > 30**	**One Way ANOVA**	
		No. = 21	**No. = 21**	**No. = 21**	**F**	**P-value**
Abdominal sample viability (%)	**Mean±SD**	97.84 ±1.14	97.79 ± 1.36	97.91 ± 1.37	0.081	0.932
	Range	96 – 99.5	95 – 100	96 – 100		NS

There is highly significant negative correlation between the BMI and the stromal cells **population** and not such correlation with **viability**.

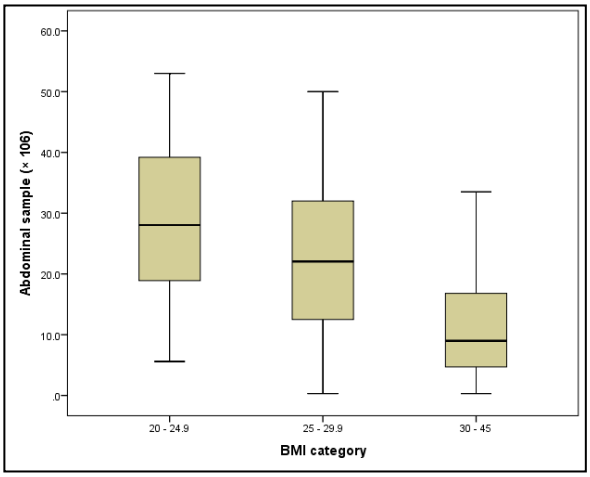

Fig. (1): Box-Plot Chart showing yield of stromal cells harvested from abdomen between Groups regarding BMI

Table (3): Comparison between yield of stromal cells in different obese patient class for each 25 ML lipoaspirate.

Population		**Obese class I**	**Obese class II**	**Obese class III**	**Kruskall-Wallis test**	
		No. = 21	**No. = 17**	**No. = 4**	**K**	**P-value**
Abdominal sample ($\times 10^6$)	**Median (IQR)**	11.2 (5.4 - 21)	6.6 (2.9 - 10.3)	10.3 (7 - 14.2)	4.012	0.136
	Range	0.9 - 36	0.3 - 15.3	4.8 - 31.5		NS

*The previous table shows that there were no statistically significant differences when there was increase in the BMI of **obese** patients regarding stromal cells population*

Table (4): Comparison between viability of stromal cells in different obese patient class

		Obese class I	**Obese class II**	**Obese class III**	**One Way ANOVA**	
		No. = 21	**No. = 17**	**No. = 4**	**F**	**P-value**
Abdominal sample viability (%)	**Mean ±SD**	98.10 ±1.24	97.80 ± 1.66	97.63 ± 1.03	0.246	0.783
	Range	96.2 – 100	96 – 100	96.5 – 98.5		NS

The previous table shows that viability of stromal cells were not be affected in different obese patient classes.

Table (5): Spearman correlation between BMI and studied samples regarding stromal cells population

	BMI	
	r	**p-value**
Abdominal sample (× 10^6)	-0.503**	0.000 HS

P > 0.05: NS, (non significant) P < 0.05: S (significant) P < 0.01: HS (highly significant)

The previous table shows that there was highly statistically significant negative correlation found between BMI and the population of stromal cells in different harvested samples.

Table (6): Spearman correlation between BMI and studied samples regarding stromal cells viability

	BMI	
	r	**p-value**
Abdominal sample viability (%)	-0.013	0.908 NS

P > 0.05: NS, (non significant) P < 0.05: S (significant) P < 0.01: HS (highly significant)

The previous table shows that there was statistically non significant correlation found between BMI and the stromal cells viability.

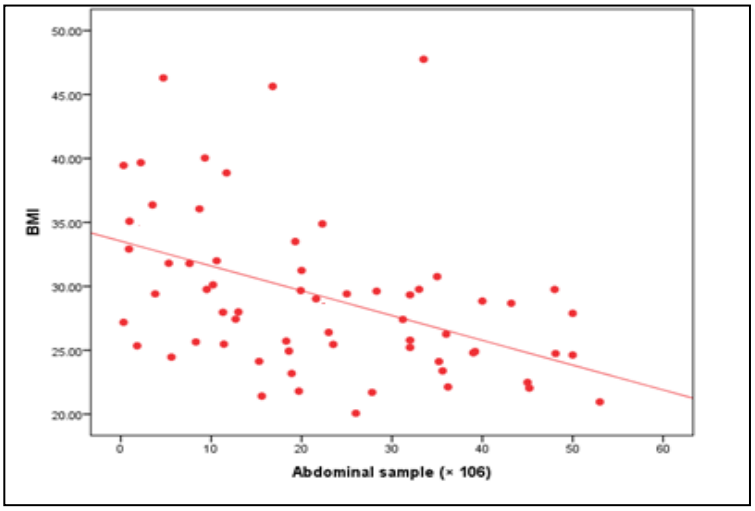

Fig (2): correlation between BMI and the abdominal samples

DISCUSSION

Stem cell technology is a developing field. Therefore, it is of utmost importance to 'track' stem cells to be understand its behavior to enable us to maximize their role in regenerative medicine. [8]

Autologous adipose tissue is considered nowadays as effective and efficient tool for augmentation of soft tissue and restoration of its volume loss in both aesthetic and reconstructive purposes. The idea of autologous adipose tissue transfer and grafting is an ideal one as it carry the advantages of being easily performed procedure in addition to minimal morbidities of harvest and potentially large donor site. The capability to avoid the usage of allogeneic or alloplastic constituents with all their potential drawbacks as

infection, antigenic, and immunologic reactions is also of great advantage.

The factors that affect the viability of the fat grafting were analyzed by many researches. Most of these studies were concerned with the mature adipocytes. [19-22]

Although the adipose derived stem cells (ADSCs) are supposed to be a favorable precursor for use in regenerative medicine, as it can be enzymatically isolated and concentrated, the literature data are scarce concerning the influence of different parameters e.g BMI index on ADSCs.

According to many studies that conducted in that field for example Rohrich et al; [23] their conclusion was that the site of harvest doesn't affect either the population or the viability of the adipose cells after comparing the thigh , , flank, abdomen , and medial knee regions. Von Heimburg et al [24] 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).In other reports such as Faustini, [25] it was shown that ADSCs yield from the abdominal region in males is more significant. The conclusion from these data appears likely that the choice of donor site exhibit a minimal role in the yield and viability of ADSCs. So, selecting a site would be based on simplicity and safety of access and patient choice. So we harvested fat from abdominal region only in all cases.

The use of either mechanical or manual suction proved to have no significant influence on the yield or viability of the ADSCs. [26] Gonzalez et al. [27] showed better adipocytes and preadipocyte viability when adipose tissue was harvested at lower negative pressure, and Mojallal A et al.[28] concluded that the quantity of ADSCs affected by the negative pressure applied during adipose tissue harvesting. So, they used the manual lipoaspiration technique for fat harvesting.

Effect of age on the proliferation capacity of mesenchymal stromal cells in both human and mouse have been studied. [29] However the effect of age on the ADSCs has been partially studied. Reports dealing with the effect of age, on yields and proliferation rates differ greatly in their conclusions. For example, the study that was conducted by Yu G et al [30] conclude that there is a positive correlation between cell yield and age of the patient. Buschmann J [31] study also report a significantly higher ADSCs cell yield of donors aged 38-44 years compared with older donor's ages > 45 years. while Faustini M et al [32] report higher cellular ADSCs yields for female donors > 45 years of age compared with female donors <35 years of age .Girolamo LD et al, [33] reported 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) Nonetheless, younger donors (20 year olds) revealed a two fold increase, which was, however, statistically insignificant. So we choosed our patients in middle ages (31-50) according to Anuradha Yarlagadda et al 2015 classification of age groups. And we found in our practice that most of patients seeking lipotransfere in this age group.

As regard the impact of BMI on the ADSCs 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, [34] reported a negative correlation between ADSCs concentration and BMI. A comparable significant negative correlation between cell yield and BMI was revealed by Van Harmelen et al. [35]

Yu [36] reports a positive correlation of ADSCs yield and BMI. Other studies determined no significant influence of BMI on cell yield. For example, Yoshimura K et al [37] , Buschmann J et al. [38] and Mojallal A et al [39].

In summary, human adipose tissue considered to be an abundant and accessible source of adipose-derived adult stem cells (ADSCs) for research purposes. The ADSCs show a reproducible and consistent phenotype based on cell viability, yield. The reproducibility and consistency of these primary human cells support their significance as an ideal adult stem cell model. Many factors affect the yield of these cells as BMI, sex, age and harvesting sites…. Further studies needed for more understanding the effects of different factors on the viability and population of ADSCs, And In the future we suggest to follow 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 ADSCs from different BMI index.

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