Value of adding autologous adipose-derived stem cells to intranasal submucosal fat implant for management of empty nose syndrome

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Background
Empty nose syndrome (ENS) is an iatrogenic disorder caused by too much nasal turbinate resection. Stem cell therapy can be used to repair, replace, or restore the biological function of a damaged tissue or organ.
Aim
To evaluate the role of adding autologous adipose-derived stem cells (ADSCs) therapy to intranasal submucosal fat implant for management of ENS and to compare its efficacy and safety to improve nasal functions in patients with ENS.
Patients and methods
Fifty-two patients having ENS were randomly distributed in two equal groups: group I was subjected to endoscopic intranasal submucosal fat implant injection and group II was subjected to intranasal submucosal fat implant with ADSCs injection at the site of inferior turbinate stump. Subjective evaluation was done by reviewing the SNOT-25 test, whereas objective evaluation was done by nasal endoscopy and nasal clearance test. Histopathological examination and reverse transcription-PCR were done to assess mucosal regeneration.
Results
Postoperative objective evaluation by anterior rhinoscopy and nasal endoscopy showed rapid healing with no signs of implant infection, rejection, or allergic reaction in both groups. Both groups experienced a statistically significant improvement in both SNOT-25 and mucociliary clearance tests after surgery. There was a positive statistical significant between the two groups from 6 months postoperatively. Both histopathological examination and reverse transcription-PCR showed evidence of mucosal regeneration in group II patients by detection of mucin-4 and lysozyme expression in regenerated nasal mucosa.
Conclusion
Adding ADSCs to intranasal submucosal fat implant augments the results and durability of improvement and also restores the anatomical and physiological functioning of nasal mucosa.

Keywords:
adipose-derived stem cells, empty nose syndrome, stem cell, turbinectomy

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Introduction

Empty nose syndrome (ENS) is an iatrogenic disorder characterized by a patent airway but has a subjective sense of poor nasal breathing [1]. Patients are diagnosed as having ENS when they have certain characteristic symptoms such as an evidence of previous nasal turbinate surgery, with improvement of their symptoms with cotton test [2]. ENS affects the normal breathing function of the nasal cavity as patients with ENS having mucosal dryness, nasal congestion, facial pain, headache on inspiration, and excessive crusting with discharge; in addition, the severity of these symptoms varies substantially among patients [3,4].

Management of ENS may be conservative with meticulous nasal hygiene and frequent intranasal irrigation to minimize the crusts and restore nasal hydration. Surgery would rebuild the internal nose to narrow the airway to provide more nasal resistance and to allow the tissue to be more moisturized by reducing airflow [5]. Various materials have been used for nasal mucosal expansion, including autologous materials (e.g. bone, cartilage, and fat) and biomaterials (e.g. teflon, plastipore, alloderm, and hydroxyapatite). Injectable materials are limited in the amount of bulk they can provide, they tend to be resorbed, and the nasal mucosa may rupture with thick injection [6].

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Cell therapy can be used to repair, replace, or restore the biological function of a damaged tissue or organ. So, the use of stem cells in cell therapy is being studied in several areas of medicine. Stem cells are undifferentiated cells capable of auto-renewing and differentiating into progenitor or precursor cells of one or several specific cell types [7].

Adult stem cells can be collected from almost any tissue; however, adipose tissue appears to be a remarkable source of mesenchymal stem cells, because adipose-derived stem cells (ADSCs) are easily isolated from a section of whole fat or lipoaspirate, which means a less aggressive and painful procedure is necessary to obtain the cells [8]. *In vitro*, ADSCs can differentiate along multiple pathways, including osteogenic, chondrogenic, adipogenic, myogenic [9], and neurogenic lineage [10] and endothelial differentiation [11]. Moreover, they secrete many cytokines and growth factors to promote neoangiogenesis and endothelialization in tissues [10]. The cytokine profile of ADSCs contains large amounts of vascular endothelial growth factor, transforming growth factor β, platelet-derived growth factor, placental growth factor, and basic fibroblast growth factor, which explains their impressive angiogenic capacity and their ability to induce tissue neovascularization [12].

The aim of this study is to evaluate the use of autologous ADSCs therapy with intranasal submucosal fat implant for management of ENS and to compare its efficacy and safety to improve nasal function of patients with ENS.

Patients and methods

Patients were recruited from the Otolaryngology Clinics of Tanta University Hospitals, Egypt. The study protocol and consent forms were approved by the Research Ethical Committee, Faculty of Medicine, Tanta University.

Inclusion criteria

Patients with a clinical and radiological diagnosis of ENS and aged between 18 and 60 years were included in the study.

Exclusion criteria

Patients with comorbidities (chronic diseases as heart diseases, liver or renal impairment, diabetes mellitus, sepsis, and malignancy), pregnancy, lactation, significant psychological problems, children under 18 years of age, inability to tolerate surgery with general anesthesia, primary atrophic rhinitis, nasal infection or allergy, and patients with severe septal deviation or septal perforation to exclude preexisting surgical variables were excluded.

Study design

This is a prospective randomized controlled study carried out from July 2016 to January 2018 (~18 months), including 52 patients with a clinical diagnosis of ENS. The patients signed the informed consent to be included in the study. These patients were randomly divided into two equal groups: group I (control group) included 26 patients subjected to surgical reconstructive treatment by endoscopic intranasal submucosal fat implant injection and group II included 26 patients subjected to endoscopic intranasal submucosal fat implant with autologous ADSCs injection into lateral nasal wall at the site of inferior turbinate stump.

The laboratory work in this study was performed for two groups, including routine laboratory investigations, such as complete blood count, prothrombin time, and bleeding and coagulation time; biochemical tests for fasting and postprandial blood glucose, liver function tests, and renal function tests; specific laboratory work such as preparation, isolation, and immunophenotyping of ADSCs; and reverse transcription (RT)-PCR. All laboratory work was done in Clinical Pathology Department, Tanta University Hospitals, Egypt.

Preparation of autologous adipose tissue-derived stem cells in group II

Collection of fat was done under complete aseptic condition under general anesthesia. Fat was extracted from the lower abdomen or thigh by liposuction using an aspirate needle according to tumescent technique [13] with a solution containing normal saline with 1 : 500 000 or 1 : 250 000 of epinephrine, at a ratio of 1 ml of solution per milliliter of aspirated tissue. Adipose tissue was then harvested using a 3-mm-diameter blunt tip cannula attached to a 60-ml luer lock syringe, creating a light negative pressure by slowly withdrawing the plunger in a gradual manner. Approximately, 100–150 ml of lipoaspirate was collected in a sterile container with care of humidity and contamination. Sample was transported directly to the laboratory of Clinical Pathology Department, Tanta University hospitals.

Isolation of adipose-derived stem cells [14]

Lipoaspirate (about 100–150 ml) was washed in equal volume of sterile PBS three to four times for 5 min. The

infranatant was then aspirated and discarded using a sterile pipette (leaving supernatant) to remove most erythrocytes and leukocytes. The washed adipose tissue (25 ml) was mixed with PBS to reach 35 ml, then 35 µl of collagenase type I (concentration 135 U/µl) was add in 50-ml conical tubes at 37°C for 1 h, and the mixture was shaken manually vigorously for 5–10 s every 15 min. The digested cell preparation was centrifuged at 2200 rpm for 10 min and then, the supernatant was aspirated and discarded leaving a stromal vascular fraction pellet, which was suspended in 50-ml solution of complete medium (DMEM, 44 ml) with 10% FBS (5 ml) plus 1% antibiotic (500 µl) and antifungal (500 µl)) to stop collagenase activity. Then centrifugation was performed for a second time at 2200 rpm for 10 min to separate the oil and remaining fat lobules from the stromal vascular fraction, discarding supernatant and leaving infranatant, which was filtered through a cell strainer of 70 µm removing unwanted debris and tissue fragment. Then repeat centrifugation was done at 2200 rpm for 5 min. The supernatant was discarded leaving a stem cell pellet, which was transferred into a sterile tube containing 10 ml of PBS.

Immunophenotyping of ADSCs [15] was performed by using ADSC surface marker analysis using flow cytometry to confirm cell form and activity were all normal. Cells were stained with phycoerythrin-conjugated antibodies for CD19, CD34, CD11b, CD105, CD73, CD90, CD45, and HLA-DR (Becton–Dickinson Biosciences, San Jose, California, USA) and incubated for 30 min in the dark at 4°C. The cells were washed twice in PBS, then the pellets suspended in 300 µl (2%) PBS and analyzed by using flow cytometry FACS Caliber (Becton–Dickinson Biosciences). ADSCs were positive for CD73, CD90, and CD105, and negative for CD19, CD34, CD45, and HLA-DR.

Reconstruct the turbinate

Approximately 30 ml (15 ml for each side) of fat particles were extracted from the lower abdomen or thigh by liposuction using an aspirate needle as described before.

All patients of both groups (52 patients) were subjected to endoscopic intranasal submucosal fat implant injection of fat aspirate. Selective infiltration of the turbinate stump was done under endoscopic guidance using 4-mm 0° endoscope (Karl Storz, Tuttlingen, Germany).

In the 26 patients of group II, additionally, the 10 ml of prepared autologous ADSCs suspension (5 ml for each side) was injected submucosal, under endoscopic guidance, in the sites a mutated turbinates via a syringe of 5 ml.

The degree of improvement of symptoms and quality of life was assessed by using SNOT-25 test [1] (Table 1). Anterior rhinoscopy, endoscopic examination, and nasal mucociliary clearance (saccharine test) all were done preoperatively and at 1, 3, 6, 12, and 18 months postoperatively. Moreover, tissue biopsy from the site of inferior nasal turbinate stump was taken before and 6 months after fat injections for histopathological examination and RT-PCR for nasal expression of lysozyme and mucin-4 (MUC4).

Histopathological examination

A punch biopsy from nasal turbinate stump mucosa ($0.2\times0.2\ cm^2$) was resected before and 6 months after the fat injections, fixed in 10% formalin neutral buffered solution, and was prepared according to standard procedure after the inclusion of paraffin and stained with hematoxylin–eosin for microscopic examinations.

Table 1 SNOT-25 nasal symptoms score for subjective assessment of patients' empty nose syndrome [1]

SNOT-25 nasal symptoms	Scoring range (0–5) from no to severe symptoms					
	0	1	2	3	4	5 (sever)
Need to blow nose						
Sneezing						
Runny nose						
Cough						
Postnasal discharge						
Thick nasal discharge						
Ear fullness						
Dizziness						
Ear pain						
Facial pain/pressure						
Difficulty falling asleep						
Waking up at night						
Lack of good night's sleep						
Waking up tired						
Fatigue						
Reduced productivity						
Reduced concentration						
Frustration/restlessness/irritability						
Sadness						
Embarrassment						
Dryness						
Difficulty with nasal breathing						
Suffocation						
Nose is too open						
Nasal crusting						

Reverse transcription-PCR

Tissue biopsy from the site of inferior nasal turbinate stump was taken before and 6 months after fat injections and the specimens were stored frozen at −70°C until RNA extraction.

RNA extraction

Total cellular RNA was extracted with the RNeasy Total RNA kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The cell lysates were mixed with an equal volume of 70% ethanol and transferred onto an RNeasy spin column, and extracted RNA was eluted with 40 µl RNAse-free water and stored at −28°C. The quantity and quality of isolated RNA was determined by absorbance at 260 and 280 nm, respectively.

Reverse transcription-PCR procedure [16]

CK7 and MUC4 primers were designed according to the sequences derived from Genebank and were synthesized by SigmaARK (Darmstadt, Germany) as shown in Table 2. Fifteen micrograms of the RNA underwent RT into complementary DNA at 37°C for 70 min in 60 µl of a volume reaction mixture that contained 150 U of RT (Superscript II; Invitrogen Life Science Technologies, California, USA), 5 µl of 50 µM oligo-dT primer, and 10 µl each of 10 mM deoxyribonucleotide triphosphate (Amersham International plc, England). The reactions were stopped by heat inactivation at 85°C for 10 min. Two microliters of each complementary DNA sample from the RT was amplified by means of RT-PCR in a volume of 50 µl containing 0.5 U of Taq DNA polymerase, 1 µl of 10 mM deoxyribonucleotide triphosphate, 2 µl of 50 mM magnesium chloride, and 1 µl of each of the 10 µM primers. PCR amplification protocol consisted of 23 cycles (for MUC4) or 30 cycles (for lysozyme) of denaturation for 1 min at 94°C, annealing for 1 min at 60°C, and extension for 1 min at 72°C, and final extension was performed at 72°C for 10 min and stopped at 4°C. The different PCR products were identified by 2% agarose gel electrophoresis followed by ethidium bromide staining, and a PCR marker size ranged from 100 to 1000 bp was used (Cat. No. P1473; Sigma, Darmstadt, Germany).

Table 2 Primer sequences of lysozyme and mucin-4 for reverse transcription-PCR amplification

mRNA	Primers 5'-3'	Product size (bp)
Lysozyme	F: CTCTCATTGTTCTGGGGC R: ACGGACAACCCTCTTTGC	350
MUC4	F: TTCTAAGAACCACCAGACTCAGAGC R: GAGACACACCTGGAGAGAATGAGC	466

MUC4, mucin-4.

Statistical analysis

Statistical analysis was conducted by using SPSS 17.0 software (SPSS Inc., Chicago, Illinois, USA). Accordingly, parametric analysis testing was done. Comparison between two groups was done by applying mean±SD, *t* test, analysis of variance *F*-test, and χ^2 for data. In all of the statistical analysis, *P* value less than 0.05 was regarded as statistically significant.

Results

This study was carried out on 52 patients with a clinical diagnosis of ENS who were randomly distributed in two equal groups. Twenty-six patients included in the group I (16 males and 10 females) were subjected to surgical reconstructive treatment by endoscopic submucosal intranasal fat implant (served as a control group), whereas the other 26 patients included in group II (18 males and eight females) were subjected to endoscopic submucosal intranasal fat implant with autologous ADSCs into lateral nasal wall at area of inferior turbinate.

Objective evaluation by anterior rhinoscopy and endoscopic examination showed that the main preoperative findings were wide nasal cavities, nasal crusting, and lack of turbinate tissue. After implantation, there was marked objective improvement regarding nasal crusting, which nearly disappears. Postoperatively anterior rhinoscopy and endoscopic nasal examination showed rapid healing with no signs of implant infection, rejection, or allergic reaction in both groups. The nasal cavities of patients were visibly more erythematous, secreted more mucus, and had fewer scabs. In two cases of group I, some degree of shrinkage or reduction of the bulk made by the graft was observed 3 months postoperatively but remains stationary after that, and it has no significant effect on the subjective outcome.

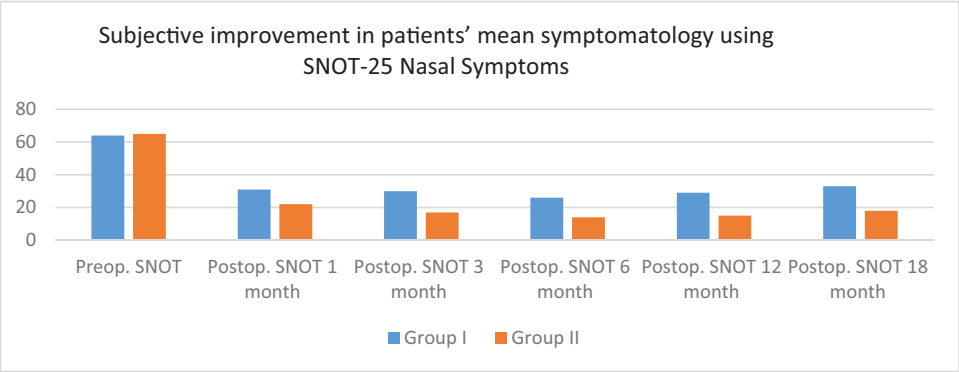
Table 3 and Chart 1 showed the mean SNOT-25 scores before and after implantation (at 1, 3, 6, 12, and 18 months postoperatively). Both groups experienced a significant improvement in the SNOT-25 test after surgery ($P<0.001$). There was a statistical evidence for a positive significant difference between the two groups ($P<0.001$) from 6 months postoperatively up till end of the study at 18 months. Chart 2 shows the most bothering patients' symptoms preoperatively in this study.

Table 3 SNOT-25 scores mean±SD before and after postoperative implantation (at 1, 3, 6, 12, and 18 months postoperatively)

SNOT-25 score	Group I (fat)	Group II (fat and stem cells)	*t* test		χ^2	
			T	*P* value	χ^2	*P* value
Preoperative SNOT-25 score (mean±SD)	64.2±12.4	65.1±12.8	1.362	0.557	**8.364**	**0.001***
Postoperative SNOT-25 score (mean±SD) (months)						
1	31.2±11.1	22.4±10.5	3.787	0.102		
3	30.4±12.3	17.3±13.4	5.366	0.09		
6	28.3±10.5	14.3±12.6	7.837	0.003*		
12	29.4±13.2	15.1± 14.2	6.925	0.001*		
18	33.3±14.3	18.1±13.2	9.075	0.0001*		
ANOVA						
F	9.763	7.945				
P value	0.111	0.0001*				

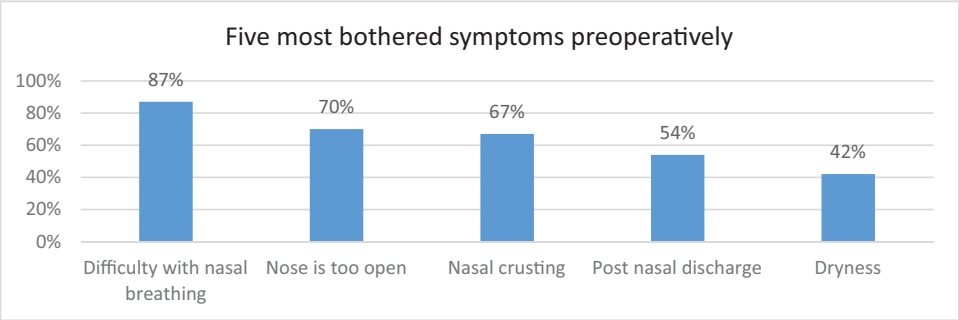
ANOVA, analysis of variance. **P*-value ≤0.05 is significant.

Chart 1



Subjective assessment of patients preoperative and postoperative by mean±SD of SNOT-25 scores.

Chart 2



Five most bothered in all patients' symptoms of both groups preoperatively.

Mucociliary clearance assessments showed a statistical improvement of saccharine clearance times in both study groups. There was a significant statistical difference between the two groups ($P<0.001$) from 6 months follow up till end of the study at 18 months (Table 4).

Histopathological examination for the hematoxylin–eosin-stained smear of nasal mucosa stump showed a satisfactory reconstruction of the mucosa and submucosa of the turbinate after 6 months from the injection of fat and ADSCs in group II more than group I and also when compared with the preoperative checkup (Fig. 1).

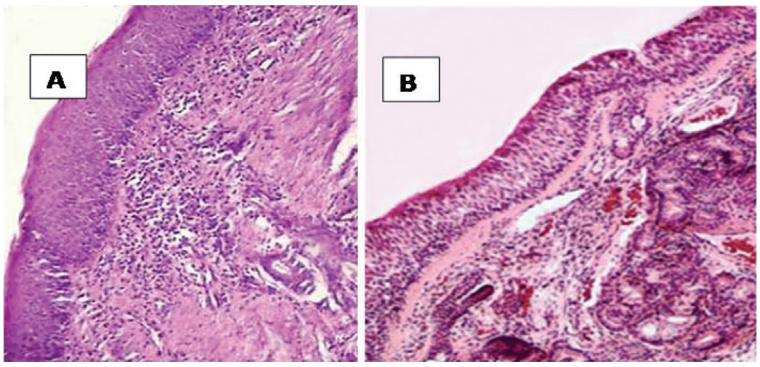
Evaluation of the function of nasal mucosa of the inferior turbinate preoperatively and postoperatively after reconstruction with and without adding ADSCs by RT-PCR.

Table 4 Objective assessment in patients' mean mucociliary clearance times preoperatively and at 3, 6, 12, and 18 months postoperatively

Saccharine clearance times	Group I (fat) (s)	Group II (fat and stem cells) (s)	*t* test		χ^2	
			t	*P* value	χ^2	*P* value
Preoperatively (mean±SD)	1645.33±1121.63	1649.31±1069.93	0.561	0.243	7.325	0.001*
Postoperatively (mean±SD) (months)						
1	1541.33±1011.54	1500.31±1069.33	0.998	0.137		
3	1457.13±978.23	1436.05±459.45	2.356	0.211		
6	1314.03±479.45	1139.03±409.75	5.137	0.001*		
12	1396.00±942.87	1105.033±395.35	3.125	0.000*		
18	1480.00±1044.28	1115.46±339.48	2.075	0.0001*		
ANOVA						
F	6.355	4.585				
P value	0.351	0.001*				

ANOVA, analysis of variance. **P*-value ≤0.05 is significant.

Figure 1



Light microscopic examination for H&E staining of biopsy from nasal mucosa stump at the site inferior turbinate (A) and from regenerated nasal mucosa after 6 months of fat implant with ADSCs injection (B) in group II. A) Nasal mucosa was infiltrated with lymphocytes and neutrophils with depletion of submucosal glands in stroma before injection of ADSCc. B) Complete re-epithelialization of the mucosal surface of the turbinate with reduction of the inflammatory part of the submucosa, numerous mucous glands, thickening of epithelium, and hyperplasia of blood vessels are seen after 6 months with fat & ADSCs in group II.

Table 5 Lysozyme expression preoperatively and 6 months postoperatively in nasal mucosa by reverse transcription-PCR

	Group I (*N*=26) [*n* (%)]		Group II (*N*=26) [*n* (%)]		χ^2	
Lysozyme expression	Positive	Negative	Positive	Negative	χ^2	*P* value
Preoperatively (mean±SD)	3±11.5	23±88.5	2±7.7	24±92.3	1.589	0.532
6 months postoperatively (mean±SD)	4±15.4	22±84.6	21±80.8	5±19.2	9.224	0.001*
χ^2						
χ^2	1.333		4.823			
P value	0.989		0.001*			

**P*-value ≤0.05 is significant.

There was an extensive positive expression of both lysozyme (80.8%) and MUC4 (88.5%) in the regenerated nasal mucosa of inferior turbinate after 6 months of ADSCs injection with fat implant in group II more than group I having fat implant only. Expression of lysozyme and MUC4 in the newly regenerated epithelial and mucosal cells was highly positive statistically significant between the two groups and in-between each group before and after 6 months of fat implant in group I and injection of ADSCs with fat implant in group II as shown in Tables 5 and 6, Figs 2 and 3

Discussion

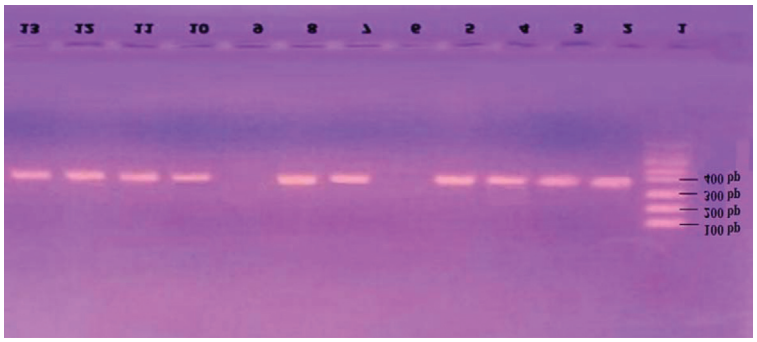
Surgery is the most frequent procedure employed in treatment of inferior turbinate hypertrophy. Too much turbinate resection may result in a wide nonfunctioning nose that have been called ENS [17]. ENS is mainly a subjective problem that reduces the quality of life, in which the main distressing symptoms are paradoxical nasal

Table 6 Mucin-4 expression preoperatively and 6 months postoperatively in nasal mucosa by reverse transcription-PCR

MUC4 expression	Group I [*n* (%)]		Group II [*n* (%)]		χ^2	
	Positive	Negative	Positive	Negative	χ^2	*P* value
Preoperatively (mean±SD)	3±11.5	23±88.5	2±7.7	24±92.3	1.995	0.684
6 months postoperatively (mean±SD)	6±23	20±77	23±88.5	3±11.5	5.859	0.001*
χ^2						
χ^2	2.461		7.952			
P value	0.186		0.001*			

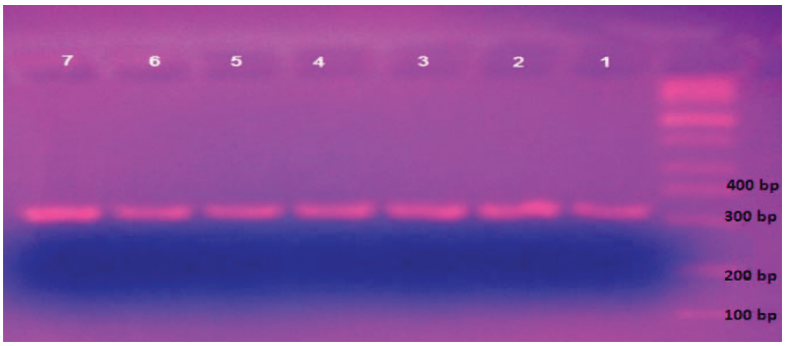
MUC4, mucin-4. **P*-value ≤0.05 is significant.

Figure 2



Agarose gel electrophoresis of the amplified product MUC4 at 466 bp compared with 100bp ladder molecular weight marker in group II after 6 months of fat implant with ADSCs injection.

Figure 3



Agarose gel electrophoresis of the amplified product Lysozyme at 350 bp compared with 100 bp ladder molecular weight marker in group II after 6 months of fat implant with ADSCs injection.

obstruction, nasal mucosal dryness, air hunger, crusting, altered nasal discharge, easy fatigability, and psychological instability. Medical treatment is commonly useless and unsatisfactory. The main aim of the surgery is to rebuild and augment the remaining turbinate stump to recover the nasal airflow resistance again [18].

A lot of materials have been used to reconstruct the ENS patient's deficient anatomy including auto-grafts (cartilage, bone, and fat), allograft (alloderm), and biomaterials such as plastipore and silastic [6]. Moreover, some injectable materials like collagen, PRP, and fat have been tried but none of them have proved to be ideal in restoring anatomy and function of the nose [19]. These grafts could be able to restore the shape of the turbinate but failed to restore the advanced function of the nasal epithelium including humidification and warming the inspired nasal airflow. With the recent advances in stem cell therapy, the hope has been renewed to use this technique for restoring the functioning nasal epithelium in those miserable patients.

The new regenerative medicine-based treatments, including extracellular matrix, platelet-rich plasma, platelet-rich lipotransfer, fat graft, and ADSC may be used for ENS treatment [20]. Stem cells are characterized by their properties of regenerative, self-

renewal, multipotency, and anti-inflammatory potential. ADSCs include mesenchymal stem cells that can differentiate into multiple lineages of mature cells that contain various types of cells, including stem cells, hematopoietic cells, smooth muscle cells, endothelial cells, immune cells, and other cell components [21]. The ADSC is an attractive therapeutic method given its safety, easy access, cells are available in large quantities, and rapid proliferation with minor tissue damage after transplantation [22].

In this study, 26 patients were subjected to submucosal intranasal fat implant (group I), whereas other 26 patients received submucosal intranasal fat implant with autologous ADSCs into lateral nasal wall at area of inferior turbinate. Subjective evaluation was done by reviewing the SNOT-25 test. The symptoms that patients reported as most troubling before implantation were fatigue, facial pain or pressure, dryness, and nose is too open; after implantation, the most common persistent concerns were facial pain or pressure and postnasal drip. The level of dryness subjectively improved in most of the patients. Both groups experienced a significant improvement after surgery. Regarding SNOT-25 evaluations, there was no statistical evidence for a significant difference between the two groups in the first 3 months of the study, but thereafter, a greater efficacy and improvement has been observed in patients of group II more than group I after 6 months postoperatively. Accordingly, there was a significant statistical difference between the two groups from 6 to 18 months postoperatively. The improvement in group II appears to be owing to the smaller quantity of intranasal crusting, better air canalization, and an improvement of the function of the mucosal surfaces of the turbinate. Our results lead us to clarify the greater efficacy of reconstruction of inferior turbinate by adding ADSCs to fat implant in group II, compared with the sole fat reconstruction in group I.

A similar observation has been noticed with the results of mucociliary clearance test where there was an improvement in the function of the mucosal surfaces of the turbinate after the reconstruction with adding of ADSCs to fat, and this improvement sustained during the whole study in group II patients, which prove that adding ADSCs to fat implant augments and maintains the results in group II.

In cases of ENS, there was damage of the mucociliary clearance with mucosal atrophy that represents the most irritable and tedious element to the quality of life. So, our study recorded that the association of surgical fat implant and ADSC injection has resulted in being an effective management of ENS to recover the volume and restore functionality of the damaged or amputated nasal mucosa. This leads us to conclude that there is a restoration of nasal neovascularization where there has been a volumetric increase with the regenerative power of ADSCs that led to a subjective and objective improvements.

These results were in agreement with Xu *et al*. [21] who reported that ADSCs developed a polygonal cobblestone shape, characteristic of human epithelial cells, and improved the function of epithelial cells by upregulating the expression of specific epithelial markers, such as cytokeratin 7, 14, and 19, which consequently improved the nasal mucosa function in patients with ENS.

Moreover, Kim and colleagues, reported that ADSCs had the power to secrete large amounts of cytokines and growth factors, which explains their impressive angiogenic capacity and their ability to promote neoangiogenesis and endothelialization in tissues.

In this study, the histopathological examinations of nasal biopsy, obtained preoperatively, and at 6 months postoperatively in both groups, showed that there was a significant difference before and after 6 months in group II, where there was a complete re-epithelialization of the mucosal surface of the turbinate with reduction of the inflammatory part of the submucosa in the areas subjected to a reconstruction by fat implant with adding ADSCs. However, in group I, nasal biopsy showed that there was no difference before and after 6 months postoperatively, where the nasal mucosa was infiltrated with lymphocytes and neutrophils with depletion of submucosal glands in stroma.

Mucins are high-molecular-weight glycoproteins with diverse biological functions and they represent the major component of mucous secretion [23]. A total of nine mucin genes, MUCl, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, and MUC8, have been identified. In-situ hybridization has indicated that seven mucin genes, MUCl, MUC2, MUC4, MUC5AC, MUC7, and MUC8, were expressed in normal and vasomotor inferior turbinates [24]. MUC1 and MUC4 are the predominant membrane bound mucins present on epithelial cell surfaces. The best membrane bound mucin is MUC4, which is engaged in interaction with signal transduction and cell survival [23]. The

function of membrane bound mucins is regulation of the airway epithelium and mucin production as mucins are the major component of mucous secretion. Mucins are produced by cells in the epithelial layers and submucosal glands and MUC4 mucins are not synthesized by the goblet cells or the mucous cells in submucosal glands but by the ciliated cells [25].

Lysozyme is a stable, low-molecular-weight enzyme that attacks the mucopeptides of bacterial cell walls and has been found in the lining cells and secretions of several types of mucosa as nasal mucosa [26]. As lysozyme belongs to the normal constituents of nasal secretion, we performed an immunohistochemical study using the indirect peroxidase-antiperoxidase method of Fukami *et al.* [27] to localize LM in the nasal respiratory mucosa, and it was clinically important to clarify that lysozyme, one of the more important serous secretions, and was secreted along with mucin. Lysozyme was found normally in the serous nasal glands as well as in the serous parts of mixed serous-mucous glands.

In this study, there was no statistical difference preoperatively between the two groups ($P>0.05$), where there was no detection or expression of both mucin (MUC4) and lysozyme in nasal biopsy by RT-PCR, whereas there was a significant statistical difference between two groups 6 months postoperatively, where there was an increase in the expression of mucin (MUC4) and lysozyme in the new regenerated nasal mucosa after 6 months of fat implant with ADSCs injection in group II ($P<0.001$), whereas no expression of both mucin (MUC4) and lysozyme in nasal mucosa stump after 6 months of fat implant only in group I.

The new clinical experience of the augmentation of nasal surgery with the regenerative therapy by adding ADSCs to fat implant in this thesis has shown the greater efficacy and safety in the processes of the mucosal regeneration and its functionality with the activation of cellular proliferation and gain of volume. The clinical effects of the injection of ADSCs in the implanted fat can promote the regenerative processes, angiogenesis, revascularization, and cellular proliferation, with production of fibroblasts and collagens in the damaged nasal mucosa [28]. ADSCs have many properties, including a wide range of sources, easy access, minor tissue damage after injections, rapid proliferation, a stable phenotype, and heredity with low immunogenicity [29], and have the potential to be differentiated into osteoblasts, chondrocyte, adipose cells, vascular endothelial cells, and epithelial cells [30,31]. ADSCs expansion *in vitro* can increase survival rate of transplanted cells, decrease rate of absorption, liquefaction and improve the revascularization of transplanted cells [29].

In this study, we are evaluating possible anatomical, physiological, and functional improvements in ENS after fat implant with and without ADSCs injection up to 18 months postoperatively and the sustainability of such improvement during the study. It is clearly obvious that adding stem cell (ADSCs) to fat has a great role in restoration of normal anatomical and functional nasal respiratory mucosa and improves the quality of life of these patients proved by the objective and subjective clinical evaluations in addition to the histopathological examinations (hematoxylin–eosin) and RT-PCR detection of MUC4 and lysozyme in the renewed nasal mucosa.

Conclusion

Using submucosal intranasal fat implant for surgical treatment of ENS is safe, effective, and relatively simple to perform. Adding autologous ADSCs to intranasal submucosal fat implant further augments the results and durability of improvement, also restoring the anatomical and physiological functioning nasal mucosa.

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Conflicts of interest

There are no conflicts of interest.

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