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ORIGINAL ARTICLE

Diode laser–assisted inferior turbinoplasty in resistant cases of allergic rhinitis: a clinical and histopathological study

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Abstract

Chronic nasal obstruction owed to inferior nasal turbinate hypertrophy is one of the most common problems encountered in rhinology. When medical management fails, surgical reduction of hyperplastic inferior nasal turbinate is often used. Diode laser is appropriate for the use in the nasal turbinate. This study was designed to define the histopathologic changes in the inferior turbinate post diode laser turbinoplasty and evaluate the outcome in patients having allergic inferior turbinate hypertrophy that has not responded to the medical treatment. A prospective single-cohort study was carried out. Under general anesthesia, 18 patients underwent inferior turbinoplasty using diode laser 980 nm in the contact mode submucosally under guidance of a 4-mm nasal endoscope 0°. Inferior turbinate mucosa biopsy specimens were taken at the time of surgery, and after 3 months, they were histopathologically examined with assessment of the patients' symptoms. At 3 months postoperatively, histopathologic assessment demonstrated marked structural changes in diode laser–treated inferior turbinates including the predominance of fibrous tissue with diminution of seromucinous glands, venous sinusoids, and inflammatory cell infiltrate. Concurrently, 16 patients (89%) had no nasal obstruction, 15 patients (83%) had moderate-to-good improvement of rhinorrhea, whereas 13 patients (72%) had moderate-to-good improvement of sneezing. Diode laser produces histopathologic changes in the inferior turbinate soft tissues, providing excellent ablation of the soft tissue with controllable performance and good hemostasis. Therefore, it is a safe, minimally invasive, and effective procedure in relieving nasal obstruction secondary to inferior turbinate hypertrophy as well as other symptoms of allergic rhinitis.

Keywords Diode laser · Allergic rhinitis · Inferior turbinate hypertrophy

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Introduction

Breathing well is a condition directly related to quality of life. Chronic nasal obstruction is a common subjective complaint encountered in the practice of rhinology. It affects patients throughout the day as well as during sleep and contributes to headache, olfactory disturbances, and sleep disorders such as snoring and obstructive sleep apnea. Hypertrophy of the inferior turbinate is a significant factor in chronic nasal obstruction. The most common non-infectious causes of mucosal swelling of the inferior turbinate are persistent allergic rhinitis, idiopathic rhinitis, compensatory hypertrophy resulting from a long-standing septal deviation, hormonal rhinitis, and drug-induced rhinitis due to use of decongestant medications and contraceptive pills [1–4].

Conservative treatment of inferior turbinate hypertrophy includes topical and systemic steroids, antihistamines, systemic decongestants, mast cell stabilizers, allergen avoidance, and hyposensitization as specific immunotherapy [5–7].

When medical management fails, various reduction techniques of the inferior turbinates seeking to increase the nasal airway passage have been applied such as inferior turbinectomy (partial or total), inferior turbinoplasty (out fracture, submucous resection, and microdebrider), and thermal techniques (electrocautery and cryotherapy) [8]. However, techniques which destroy the inferior turbinate mucosa lead to loss of turbinate function along with crusting and adhesion and are associated with a risk of bleeding, uncontrolled damage to the mucosa, pain, and atrophic rhinitis [9].

Therefore, there is a need for less traumatic, minimally invasive, safe, and effective method for volume reduction of hypertrophied inferior turbinate. This can be accomplished by laser surgery as it provides limited tissue trauma, less bleeding, and high patient acceptance [10].

Various kinds of lasers like CO2, KTP, neodymium:yttrium aluminum garnet (Nd:YAG), holmium:yttrium aluminum garnet (Ho:YAG), and diode are used for inferior turbinate reduction. The fundamental difference between these lasers is the wavelength of the emitted light, which provokes different interactions in between laser light and tissue. The most appropriate lasers for the use in the nasal turbinate are Ho:YAG laser and diode laser [9, 11]. Fukutake et al. [12] used laser not only to improve nasal breathing, but also to reduce rhinorrhea. Diode laser light is absorbed by water and blood. Thus, it provides excellent coagulation. Moreover, diode laser can be used in contact application offering vaporization with precise tissue cutting [9, 13].

The issue of postoperative histopathologic changes in the nasal mucosa still has not been adequately addressed. Therefore, the aims of the present study are to address the histopathologic changes in the inferior turbinates after diode laser turbinoplasty and to evaluate the outcome in patients with allergic rhinitis having refractory inferior turbinate hypertrophy.

Material and methods

A prospective single-cohort study has been conducted in the Oto-Rhino-Laryngology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt, during the period from July 2019 to March 2020. The study has been approved by the Zagazig University Institutional Review Board (IRB).

Inclusion criteria Patients of allergic rhinitis complaining of nasal obstruction due to hypertrophy of the inferior turbinates that has not responded to the medical treatment (antigen avoidance, oral antihistamines, intranasal steroid sprays, and systemic decongestants) for at least 3 months were enrolled in this study. All patients had positive prick skin testing for allergy (the allergens included dust, feather, wool, cotton, pollens, clover, hay rice dust, fungi, and maize).

Exclusion criteria Patients not fit for surgery; pregnant patients; non-allergic rhinitis patients, e.g., rhinitis medicamentosa, gustatory rhinitis, senile rhinitis, hormonal rhinitis, and vasomotor rhinitis; and patients having nasal septal deviation, polyposis, chronic rhinosinusitis, granulomas, tumors of the nose and paranasal sinuses or hypertrophied bone of inferior turbinate, and past history of inferior turbinate surgery.

A fully informed written consent was obtained from all the patients. All the patients underwent the following diagnostic protocol:

(a) Full clinical history taking,
(b) A five-point scale was used to assess the severity of nasal obstruction of the patient [14] (level 0: no nasal obstruction, level 1: no mouth breathing, level 2: occasional mouth breathing, level 3: predominant mouth breathing, and level 4: no nasal breathing). As well, subjective evaluation of the associated nasal symptoms, rhinorrhea, and sneezing was done by using the visual analogue scale (VAS). Patients rated symptoms from a score of 0 to 10 with score 0 being asymptomatic and 10 being the most severe symptom, as mild: 0–3, moderate: 4–7, and severe: 8–10,
(c) Complete ENT examination including rigid or flexible nasal endoscopy. The inferior turbinate was graded 1–4 based on the inferior turbinate classification system by Camacho et al. [15] in which the total amount of the airway space that the inferior turbinate occupied was estimated as grade 1: 0–25% of total airway space, grade 2: 26–50% of total airway space, grade 3: 51–75% of total airway space, grade 4: 76–100% of total airway space,
(d) Decongestion to rule out bony hypertrophy of the inferior turbinate,
(e) Computed tomography scanning of the nose and paranasal sinuses without contrast,
(f) The necessary routine preoperative laboratory workup.

I. The operative technique

- Under general anesthesia, after preparing and draping the patient in the usual sterile manner, a biopsy was taken from the medial surface of the anterior two-thirds of the inferior turbinate using Blakesley thru-cut forceps for histopathologic examination.
- The regular safety laser precautions were taken. Under guidance of 4-mm nasal endoscope 0°, a 600-μm quartz

fiber was inserted into the inferior turbinate submucosally along its medial surface starting at its anterior end and proceeding posteriorly. The power of diode laser 980 nm (Wuhan Gigaa Optronics Technology Co., Ltd., Model V-100) was set at 6 W and was delivered in a continuous and contact mode via the laser flexible fiber.

– Three parallel stripes were made to each inferior turbinate by withdrawing the laser flexible fiber slowly from the posterior to the anterior along its medial surface with suctioning of the smoke which happened during the procedure. The effect of diode laser appeared as blanching of inferior turbinate tissue. Additional laser application was performed on hyperplastic tissue in aerodynamic important areas.
– Then, the nose was packed with one Sofra-Tulle (a gauze dressing impregnated with an antibiotic) into each nasal cavity.

II. Postoperative follow-up

– The nasal pack was removed after 24 h, and the patients were discharged on the same day and were given topical decongestant and analgesic for 7 days and saline nasal spray for 3 months.
– Postoperatively, the patients were followed-up after 2 weeks; then every month and at every follow-up visit, rigid or flexible nasal endoscopy was performed to assess the nasal airway as regards to the patency, crust formation, and synechiae.
– After 3 months, a biopsy was re-taken from the medial surface of the anterior two-thirds of the inferior turbinate mucosa under local anesthesia by using the Blakesley thru-cut forceps for histopathologic examination with re-assessment of the patient nasal blockage using the same scale, while improvement of the associated nasal symptoms, rhinorrhea, and sneezing was scaled as no improvement: 1 point, moderate: 2–3 points, good: 4–5 points, and excellent: 6 points.

III. Histopathological study The inferior turbinate specimens were processed in the pathology department. The biopsies were fixed in 10% buffered formaldehyde and embedded in paraffin. Five-micrometer-thick sections were cut from formaldehyde-fixed and paraffin-embedded tissue blocks. Sections were deparaffinized in xylene, then rehydrated in graded alcohol, and stained with hematoxylin and eosin. The hematoxylin and eosin–stained sections were examined, and a qualitative assessment of the sections was performed to indicate the type of lining epithelium, any mucosal change, the presence of inflammation and its intensity (the percentage of non-scarred tissue involved by mononuclear cell infiltrate), the type of inflammatory cell infiltrate, the presence of fibrosis, and the population of submucosal seromucinous glands and venous sinusoids.

Table 1 The severity of nasal obstruction

The severity of nasal obstruction	No. (18)	%
Level 3 (predominant mouth breathing)	7	39%
Level 4 (no nasal breathing)	11	61%

Table 2 Preoperative grading of inferior turbinate hypertrophy

Grading of inferior turbinate hypertrophy	No. (18)	%
Grade 3	8	44.4%
Grade 4	10	55.6%

IV. Statistical analysis Data were checked, entered, and analyzed using SPSS version 23 for data processing. Data were expressed as the number and percentage for qualitative variables and the mean ± standard deviation (SD) for quantitative ones. The non-numerical data was tabulated and compared using chi-square test (X^2). The threshold of significance was fixed at 5% level (*P*-value). The smaller the *P*-value obtained, the more significant are the results. *P*-value of > 0.05 indicates non-significant results, *P*-value of < 0.05 indicates significant results, and *P*-value < 0.001 indicates highly significant results.

Results

This study included 18 patients. Their mean age was 28.7 ± 6.7 years. Ten patients were females and eight patients were males. All patients showed up for the follow-up visits. Preoperatively, 11 patients had level 4 nasal obstruction (no nasal breathing), while 7 patients had level 3 nasal obstruction (predominant mouth breathing) (Table 1). The patients had either grade 3 or 4 inferior turbinate hypertrophy (Table 2). As well, 11 patients had severe rhinorrhea (61%), whereas 9 patients had severe sneezing (50%) (Table 3). None of the patients had bleeding postoperatively. After diode laser inferior turbinate reduction, there were few isolated crusts on the inferior turbinates and the crusts have disappeared completely by the end of the 3rd month. At 2 weeks postoperatively, synechiae were noted in 3 patients (16.7%) and release of these synechiae was done in the outpatient clinic

Table 3 Preoperative associated nasal symptoms

Severity	Rhinorrhea		Sneezing	
	No. (18)	%	No. (18)	%
Mild	3	17%	4	22%
Moderate	4	22%	5	28%
Severe	11	61%	9	50%

VAS: Visual analogue scale. Score of 0 to 10 with a score 0: being asymptomatic, 10: being the most severe symptom. Mild: 0–3, moderate: 4–7, severe: 8–10

Table 4 Postoperative improvement of nasal obstruction

Improvement of nasal obstruction	No. (18)	%
Level 0 (no nasal obstruction)	16	89%
Level 1 (no mouth breathing)	2	11%

Table 5 Postoperative improvement of the associated nasal symptoms

Improvement	Rhinorrhea		Sneezing	
	No. (18)	%	No. (18)	%
No improvement	3	17%	5	28%
Moderate	5	28%	7	39%
Good	10	55%	6	33%
Excellent	0	0%	0	0 %

No improvement: 1 point, moderate: 2–3 points, good: 4–5 points, excellent: 6 points

department under local anesthesia. At 3 months postoperatively, all the patients had intact shrunken inferior turbinate and no atrophic change has been observed in any patient. Sixteen patients (89%) had no nasal obstruction (Table 4), 15 patients (83%) had moderate-to-good improvement of rhinorrhea, whereas 13 patients (72%) had moderate-to-good improvement of sneezing (Table 5).

Histopathologic assessment of the inferior turbinate biopsy specimens taken at the time of surgery revealed that the epithelial layer was mainly composed of a pseudostratified columnar epithelium with deeply situated basal cells, superficially ciliated and non-ciliated cells, and an appreciable number of goblet cells. However, areas of denuded epithelium were noted in 4 patients, whereas small areas of metaplastic squamous epithelium were encountered in 2 patients. Beneath the basement membrane, there was a lamina propria made of loose connective tissue and moderate-to-marked inflammatory cell infiltrate was detected in the lamina propria where the lymphocytes, plasma cells, and eosinophils were the prominent inflammatory cells. As well, dilated and engorged thin-walled venous sinusoids and islands of seromucinous glands of varying degree were noted throughout the submucosa (Figures 1, 2, and 3).

At 3 months postoperatively, there were marked structural changes in diode laser–treated inferior turbinates as considerable differences were noted between the preoperative and postoperative biopsy specimens (Figures 4 and 5). Although normal appearing pseudostratified ciliated columnar epithelium was present, areas of epithelium loss were found (5 patients), while small islands of squamous metaplasia were encountered (3 patients). However, such

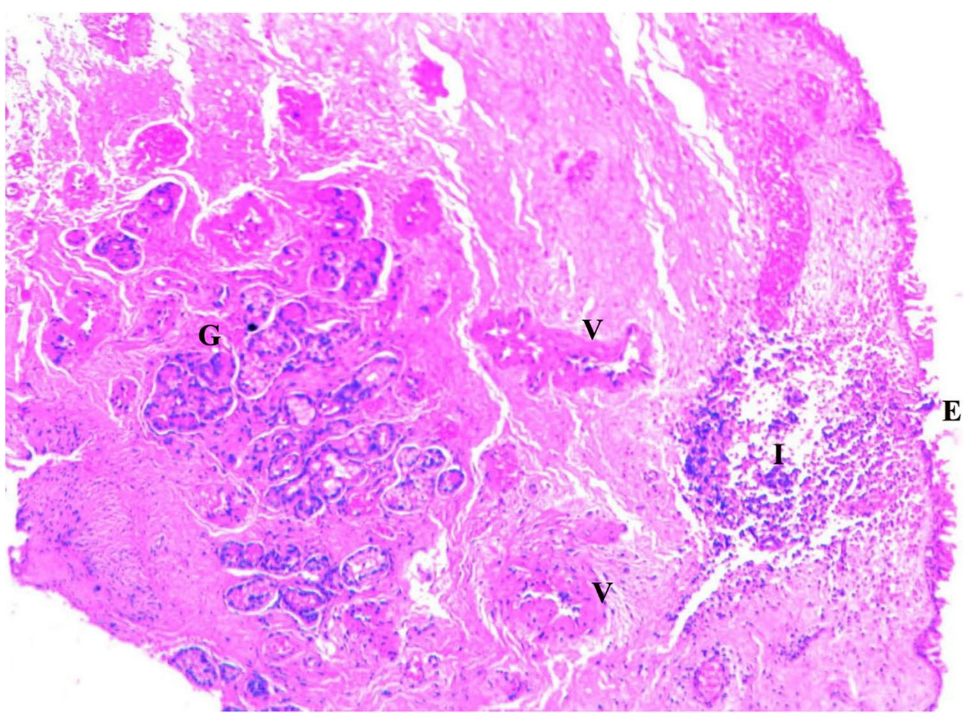

Fig. 1 A preoperative section from a patient with inferior turbinate hypertrophy showing partial loss of respiratory epithelium (E), proliferated mucous glands (G), numerous vascular spaces (V), and inflammatory cell infiltrate (I) (H&E × 100)

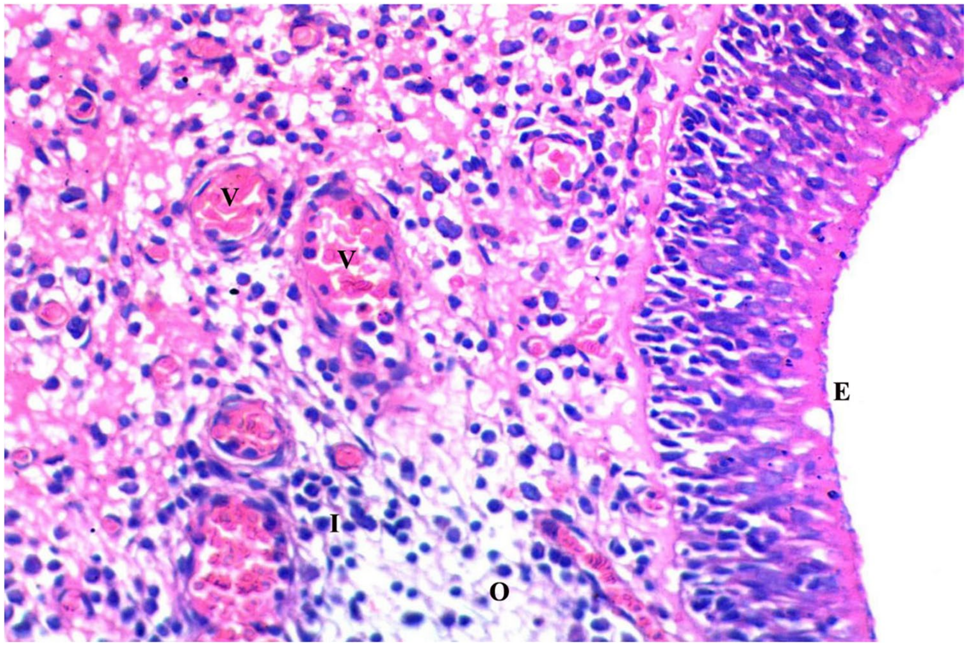

Fig. 2 A preoperative section from a patient with inferior turbinate hypertrophy showing intact respiratory epithelium (E), edema (O), numerous dilated venous sinusoids (V), and moderate inflammatory cell infiltrate (lymphocytes, plasma cells, and eosinophils) (I) (H&E × 400)

epithelium change was statistically insignificant ($P > 0.05$) (Table 6). A well-defined basement membrane was present across the laser-treated areas in all cases. However, the loose connective tissue of the normal lamina propria was replaced by varying degrees of fibrosis with dispersed islands of inflammatory cells were seen throughout the loose connective tissue, whereas the fibrotic areas were devoid of the cell infiltrate ($P < 0.0001$) (Table 7). As well, marked decrease of submucosal glands ($P < 0.0001$) (Table 8) and venous sinusoids ($P < 0.0001$) (Table 9) was denoted.

Discussion

Chronic hypertrophic rhinitis is commonly associated with perennial allergic or non-allergic rhinitis. When the inferior turbinate becomes hypertrophied and increasingly

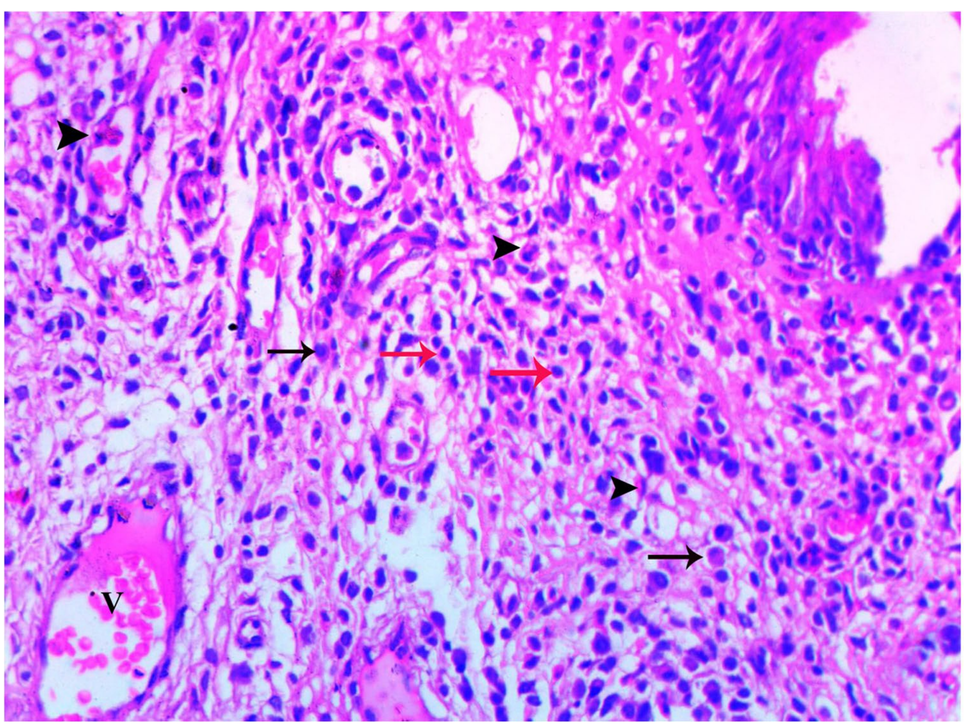

Fig. 3 A preoperative section from a patient with inferior turbinate hypertrophy showing dilated capillaries (V) and dense inflammatory cell infiltrate formed of lymphocytes (red arrow), plasma cells (black arrow), and eosinophils (arrow head) (H&E × 400)

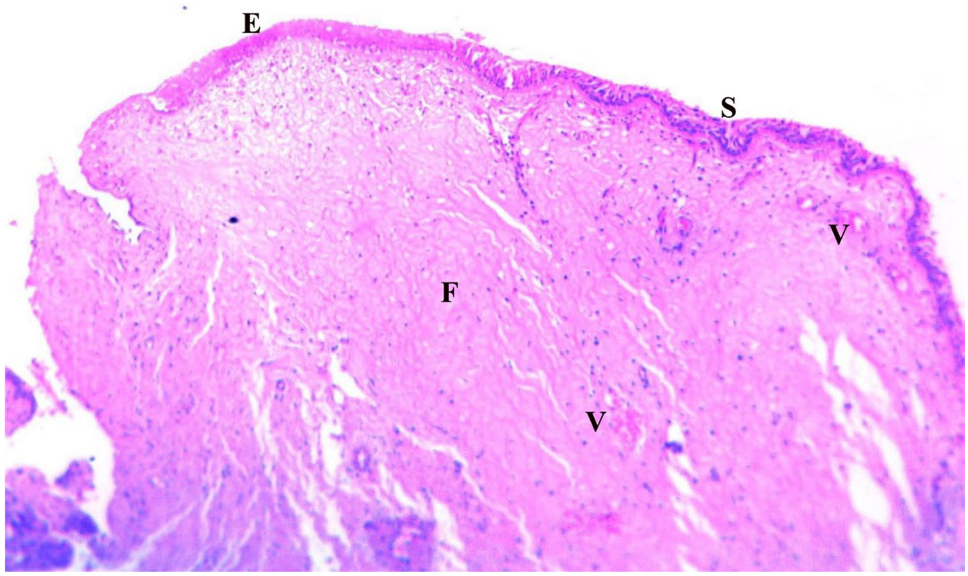

Fig. 4 A postoperative section from a patient with inferior turbinate hypertrophy showing intact respiratory epithelium (E) with area of squamous metaplasia (S), marked fibrosis (F), and scattered vascular spaces (V) (H&E × 100)

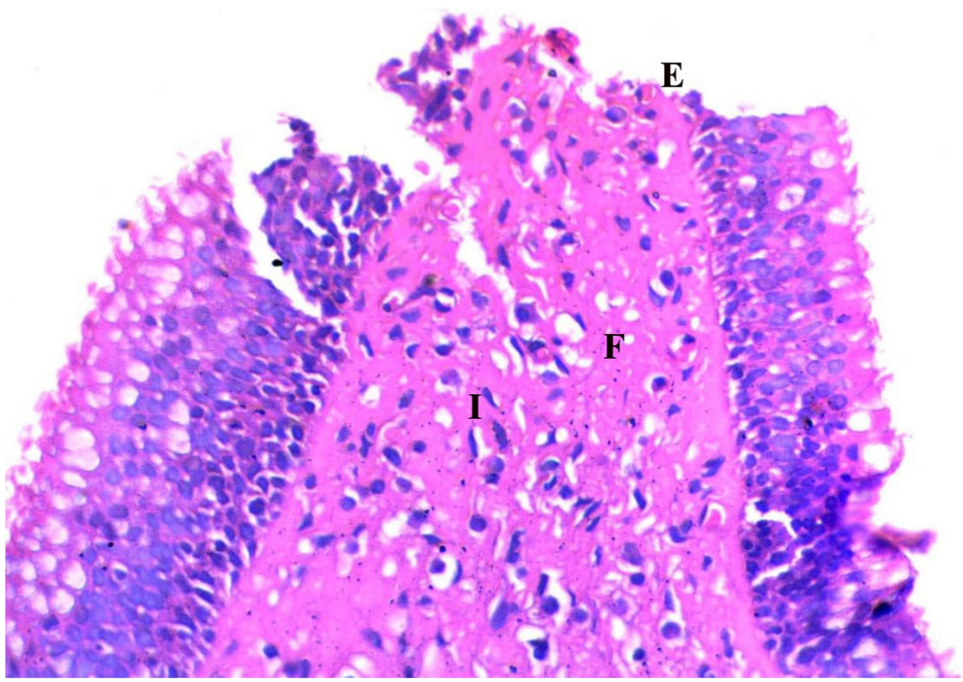

Fig. 5 A postoperative section from a patient with inferior turbinate hypertrophy showing partial loss of respiratory epithelium (E), marked fibrosis (F), and mild inflammatory cell infiltrate (I) (H&E × 400)

Table 6 Preoperative and postoperative respiratory epithelial changes

Respiratory epithelial changes	Preoperative		Postoperative		χ^2	*P*-value
	No. (18)	%	No. (18)	%		
Preserved ciliated	12	67%	10	55%	0.468	0.4939
Loss of epithelium	4	22%	5	28%	0.148	0.7
Squamous metaplasia	2	11%	3	17%	0.232	0.63

Statistically insignificant difference ($P > 0.05$)

encroaches on the airway passage, nasal obstruction occurs. Therefore, it affects the quality of life [16]. In such cases, the conservative medical therapy is considered the initial treatment. When nasal obstruction is unresponsive to the medical treatment or if the hypertrophic change of the inferior turbinate is perceived as irreversible, inferior turbinate

Table 7 Preoperative and postoperative inflammatory cell changes

Inflammatory cell infiltrate	Preoperative		Postoperative		χ^2	*P*-value
	No. (18)	%	No. (18)	%		
Mild	1	5%	14	78%	2.378	< 0.0001
Moderate	5	28%	4	22%		
Marked	12	67%	0.0	0.0%		

Statistically highly significant difference ($P < 0.0001$)

Table 8 Preoperative and postoperative seromucinous gland changes

Seromucinous gland proliferation	Preoperative		Postoperative		χ^2	*P*-value
	No. (18)	%	No. (18)	%		
Mild	0.0	0.0%	15	83%	28	< 0.0001
Moderate	6	33%	3	17%		
Marked	12	67%	0.0	0.0%		

Statistically highly significant difference ($P < 0.0001$)

Table 9 Preoperative and postoperative venous sinusoid changes

Venous sinusoid proliferation	Preoperative		Postoperative		χ^2	*P*-value
	No. (18)	%	No. (18)	%		
Mild	0.0	0.0%	13	72%	24.333	< 0.0001
Moderate	7	39%	5	28%		
Marked	11	61%	0.0	0.0%		

Statistically highly significant difference ($P < 0.0001$)

surgery should be considered. However, other etiologies for nasal obstruction should be ruled out preoperatively such as structural deformities of the nasal septum, sinusitis, nasal polyps, tumors, and adenoid hypertrophy in pediatric patients [17].

The goal of inferior turbinate surgery is to obtain an improvement in nasal breathing, while preserving the physiologic function of the turbinate and minimizing discomfort and other adverse effects. However, no technique is perfect. The variety of surgical techniques available highlights the absence of unanimity regarding the optimal technique. Techniques which destroy the turbinate mucosa lead to loss of turbinate function along with crusting and adhesions [12].

In case of allergic rhinitis, mucosal hypertrophy is the main cause for inferior turbinate hypertrophy rather than bony hypertrophy [18]. The underlying concept of laser-assisted inferior turbinoplasty is to achieve turbinate volume and surface reduction without excessive damage to the surrounding tissue that could result in excessive mucosal loss and/or bone exposure. Laser can be performed in either linear, anterior to posterior stripe, or cross-hatching method. It can be applied to either the entire turbinate or a particular focus, such as the anterior turbinate head or inferior edge [19].

In the present study, the preoperative histopathologic findings are in accordance with that of Berger et al. [16] as they reported that small islands of squamous metaplasia were encountered in 2 specimens, while areas of denuded epithelium were found in normal and hypertrophied inferior turbinates. As well, marked subepithelial inflammatory cell infiltrate comprising a mixture of lymphocytes, macrophages, plasma cells, and eosinophils was present in about two-thirds of the samples (13/20), while dilated and engorged thin-walled venous sinusoids were noted in 3 samples (15%). Our results coincide with those of Hegazy et al. [20] who mentioned that in inferior turbinate hypertrophy secondary to allergic rhinitis, lymphocytes, plasma cells, and eosinophils were the prominent inflammatory cells and there was increased number of blood sinusoids with associated rupture and hemorrhage. Also, Bhat [18] reported that 63% of the patients with allergic rhinitis showed an inflammatory cell infiltrate in the inferior turbinate specimens and eosinophils and plasma cells were the common inflammatory cells, while engorged venous sinusoids were encountered in 35.2% of the patients. Whereas Wexler et al. [21] noted that in hypertrophic inferior turbinates secondary to perennial allergic rhinitis, a moderate inflammatory cell infiltrate was seen in the lamina propria with islands of both seromucinous glands and venous sinusoids throughout the submucosa.

At 3 months postoperatively, diode laser–treated inferior turbinates displayed marked structural changes. Our post laser histopathologic results concur with those of Wexler et al. [21] as they reported a predominance of connective tissue, a notable decrease of submucosal glands, and sparse venous sinusoids in laser-treated inferior turbinates. As well, the connective tissue pattern included areas of loose stroma similar in appearance to normal lamina propria as well as fibrotic areas with tightly arranged collagen fibers and scattered inflammatory cells were seen throughout the loose connective tissue. Also, Elwany and Abel Salaam [22] mentioned that the dense fibrous stroma is characteristic of laser-treated turbinates with reduction of the number and activity of the nasal glands.

In the present study, 3 months postoperatively, improvement of the nasal symptoms was reported as 16 patients (89%) had no nasal obstruction, 15 patients (83%) had moderate-to-good improvement of rhinorrhea, and 13 patients (72%) had moderate-to-good improvement of sneezing. Our results coincide with those of Gupta et al. [23] as they reported statistically significant improvement of nasal obstruction, rhinorrhea, and sneezing 3-month post diode laser turbinate reduction. As well, our results are in accordance with that of Ibrahim et al. [24] who mentioned that diode laser turbinoplasty resulted in complete improvement of nasal obstruction in 92% of the patients, while Parida et al. [25] reported relief of nasal obstruction in 86.7% of the patients post diode laser turbinate reduction.

Also, Hoque et al. [13] mentioned that diode laser turbinoplasty resulted in moderate-to-strong subjective improvement of the nasal airflow in 70% of the patients, relief to the improvement of rhinorrhea in 59.1% of the patients, and disappearance to the improvement of sneezing in 57.9% of the patients. The discrepancy of their results to our results can be explained by the different selection of the cases as among their patients, 16 patients (53.3%) had allergic rhinitis and 14 patients (46.7%) had vasomotor rhinitis, whereas in our study, all patients had allergic rhinitis, while vasomotor rhinitis was excluded.

Berger et al. [26] mentioned that in inferior turbinate hypertrophy, the enlarged area is often localized in the medial mucosal layer of the inferior turbinate. As well, the inferior turbinate enlargement is attributed to the increased number of submucosal/seromucinous glands and/or increased number of vessels as a result of neo-angiogenesis [16, 27].

Diode laser uses high-power laser beam for vaporization, incision, or coagulation of the tissues. In the present study, diode laser is used in the contact mode and is applied to the submucosa of the entire inferior turbinate as anterior to posterior stripe. As the flexible fiber accurately delivers the beam, it produces minimal damage to the nearby areas. Moreover, the presence of a viable respiratory epithelium including goblet cells suggests that the epithelium over the laser-treated areas may provide sufficient protection and humidification even with substantial loss of submucosal glandular tissue.

Thus, according to the produced histopathologic changes by diode laser, diode laser turbinoplasty results in an inferior turbinate reduction by producing necrosis of the submucosal turbinate tissue with sparing the mucosal layer. Furthermore, the submucosal venous sinusoids and glands are replaced by sclerotic connective-like tissue, decreasing the size of turbinates. As well, the improvement of rhinorrhea can be attributed to induction of fibrosis in the highly vascular submucosa, reduction of the seromucinous glands, and incision of branches of posterior nasal nerve, whereas the relief of sneezing can be attributed to destruction of the branches of posterior nasal nerve which plays a crucial role in sneezing and hypersecretion. Consequently, the improvement of rhinorrhea and sneezing can be attributed to the change of the secretory reflex [25, 28, 29].

The present study results concur with those of Caffier et al. [30] who reported that endonasal diode laser surgery was an effective, safe, and well-tolerated treatment option for therapy-resistant perennial and seasonal allergic rhinitis providing long-lasting symptom reduction. However, the current study has limitations as the patients' symptoms were assessed subjectively and the follow-up period is relatively short. Therefore, acoustic rhinometry or rhinomanometry can be used to objectively assess the patients' symptoms and longer follow-up is recommended. As well, further studies need to be conducted to study the mucociliary clearance post diode laser turbinoplasty.

Conclusion

Diode laser in the submucosal contact mode provides excellent ablation of the soft tissue with controllable performance and good hemostasis. Diode laser–assisted inferior turbinoplasty has the advantages of preserving the mucosa for turbinate function, enhanced precision and controlled volume reduction, minimal trauma with less bleeding and crusting, and shorter duration of nasal packing and hospital stay. Therefore, it is a safe and effective procedure. The present study denotes that diode laser produces histopathologic changes in the inferior turbinate soft tissues and advocates its usefulness for inferior turbinate reduction surgery. Thus, it can be considered a valuable tool in treating allergic refractory inferior turbinate hypertrophy. The clinical benefits gained with diode laser are not only limited to nasal obstruction, but also extending to other symptoms of allergic rhinitis. To ensure the efficiency of diode laser in providing long-lasting improvement of the nasal symptoms, a longer follow-up is recommended.

Declarations

Ethics approval The Institutional Reviewer Board (IRB) of the Faculty of Medicine, Zagazig University, Egypt, has approved the study.

Informed consent A fully informed written consent was obtained from all patients included in the study.

Consent for publication This manuscript is original, has not been previously published, and is not under consideration for publication elsewhere. This manuscript has not been presented in any meeting.

Conflict of interest The authors declare no competing interests.

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