Indian J Otolaryngol Head Neck Surg
(November 2019) 71(Suppl 3):S1810–S1815; DOI 10.1007/s12070-017-1176-4

ORIGINAL ARTICLE

# Effect of Radiofrequency on DNA Damage and Oxidative Status in Patients with Turbinate Hypertrophy

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Received: 9 February 2017 / Accepted: 28 July 2017 / Published online: 4 August 2017


**Abstract** The radiofrequency devices that are used generate radiofrequency in the frequency range of 1.5 and 2.5 MHz. This study aims to demonstrate whether systematic oxidative status and DNA are influenced in this frequency range. In study, 27 patients who received radiofrequency treatment on inferior turbinate as they were diagnosed with inferior turbinate hypertrophy. DNA damage was assessed by alkaline comet assay in peripheral lymphocyte cells. Plasma levels of total antioxidant status (TAS), total oxidative status (TOS) were determined by using an automated measurement method and oxidative stress index (OSI) was calculated (OSI was calculated as: OSI = (TOS/TAS) × 100). There were increased in the OSI and TOS values on days 1 and 15 as compared to the samples taken before the radiofrequency administration. Significant decreases were seen in TAS values on days 1 and 15. As for the DNA damage, no significant differences were found on day 15 compared to the preoperative values even though there was a statistically insignificant increase on day 1. Administration of radiofrequency radiation on inferior turbinates results in increased oxidative stress in the acute period and a decrease in the anti-oxidative system. Although this effect causes a slight increase in the DNA damage in the early post-operative period, the damage is restored to the pre-operative levels on day 15. Therefore, we believe that a more conservative approach should be selected for radiofrequency treatment instead of using it routinely.

**Keywords** Radiofrequency radiation · Inferior turbinates · Oxidative stress · DNA damage

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## Introduction

Inferior turbinate hypertrophy is one of the most frequent reasons for anatomic nasal obstruction in rhinology practice. Today, for the medical treatment of inferior turbinate hypertrophy, nasal steroids, antihistaminics, systemic and local decongestants and mast cell stabilizers are used [1]. As for surgical treatment, lateralization, sub-mucosal resection, partial resection, cryo-therapy, electrocauterization, laser or radiofrequency (RF) reduction can be used. Radiofrequency ablation is one of the most preferred methods among surgical treatments [2].

Submucosal radiofrequency administration induces the ion stimulation in tissue, increases the local temperature and causes thermal damage in deep mucosa without harming the surface. In the long term, it results in submucosal fibrosis and reduced volume of the inferior turbinate [3]. This method is not cause serious complications and can be applied easily.

For radiofrequency ablation therapy, different frequencies are selected depending on the tissue where it be applied. For the RF administration, devices in the frequency range of 1.5 and 2.5 MHz are generally used. A study was done to see whether high frequency radiation administration caused DNA damage on human lymphocytes under in vitro conditions [4]. However, no studies have been conducted to find out whether radiofrequency

treatment applied on inferior turbinates and at lower frequencies had an effect on the oxidative status or not. Recent studies have demonstrated that increased oxidative stress caused DNA damage according to their results [5]. This study aimed to investigate the effects of RF administration for inferior turbinate hypertrophy on the mononuclear leukocyte DNA damage, total oxidative and anti-oxidative levels. Therefore, we were able to observe that a more conservative approach could be taken in radiofrequency administration if a significant oxidative stress and DNA damage occurred.

## Materials and Methods

### Subjects

Our study was conducted between August, 2013 and February, 2014 after the approval from the local ethics committee for clinical research was obtained. In this cross-sectional study, 27 patients who presented to the ENT service of our university for nasal obstruction and were diagnosed with inferior turbinate hypertrophy were included. The rules of Helsinki declaration were complied with in the study protocol. The study protocol was explained to all patients and their consent was received. The study protocol was approved by the Clinical Trials Ethics Committee of our university. Those who had acute or chronic sinusitis, septal deviation, asthma, used systemic steroids, nasal steroids, membrane stabilizer antihistaminic or nasal decongestant medicines and who were on immunotherapy were excluded.

Radiofrequency ablation was delivered on the patients in semi-upright sitting position after the application of cotton strip absorbed with 10% lidocaine for 5 min in the nasal cavity. Local anesthesia comprised 1–2 mL of lidocaine HCl (40 mg/mL) and adrenalin (0.025 mg/mL) (Jetosel) was infiltrated into the inferior turbinate. A concha probe was inserted into the sub-mucosa. (RadioSURG$^{R}$ 2200 2.2. MHz, Meyer-Haake GmbH Medical Innovations, Obermörlen, Germany) Radiofrequency energy was delivered to the anterior, middle, and posterior portions of the inferior turbinate.

### Blood Collection and Storage Conditions

Peripheral vessel blood samples were collected from the patients in the morning of the operation day (before the operation) 1 day after and 15 days after the operation. The blood samples of 5 mL were taken from the forearm veins of participants. The samples were immediately placed in heparinized tubes, stored in a dark place at 2–4 °C and they were processed within 2 h in order to avoid any DNA damage. Mononuclear leukocytes were isolated via centrifuge in Histopaque 1077 (Sigma). The remaining heparinized blood was centrifuged at 1500×*g* for 10 min and the plasma obtained was stored for TOS and TAS analyses at −80 °C.

### Determination of DNA Damage Using the Alkaline Comet Assay

The comet assay or the single-cell gel electrophoresis (SCGE) assay was performed by making certain modifications to the method by Singh et al. [6]. All the manipulations were performed under minimal illumination. The dried microscope slides were covered with cover slips, and viewed by fluorescence microscopy (Olympus BX51, Japan) at 200× magnification. The extent of extranuclear fluorescence was scored (by eye) in 50 random cells of each sample using a scale of 0–4 as previously described by Kobayashi et al. [7]. Scoring was as follows: 0, no tail; 1, comet tail < half the width of the nucleus; 2, comet tail equal to the width of the nucleus; 3, comet tail longer than the width of the nucleus but not twice as long; 4, comet tail > twice the width of the nucleus. This type of scoring has been shown to be as accurate that afforded by computerized image analysis [8]. All slides were coded and were scored in a blinded manner. A visual score for each class of subjects was calculated by multiplying the percentages of cells in the various comet classes by the score for that class. The total visual comet score reflecting the extent of DNA damage was the sum of scores for all five comet classes. Thus, a total visual score could range from 0 (all undamaged) to 400 (all maximally damaged) in arbitrary units (AU).

### Measurement of Total Oxidant Status

Plasma TOS was measured using a novel automated method developed by Erel [9]. Oxidants present in a sample oxidize the ferrous ion of an o-Dianisidine complex to ferric ion. Color intensity (which can be measured spectrophotometrically) is associated with the total level of oxidants present. Hydrogen peroxide is used to calibrate the assay and results are expressed in terms of micromoles of hydrogen peroxide equivalent per liter (mmol $H_2O_2$ equiv./l).

### Measurement of Total Antioxidant Status

Plasma TAS was measured using another novel automated method developed by Erel [10]. This involves production of the hydroxyl radical, which is a potent biological reactant. A ferrous ion solution (Reagent 1) is mixed with hydrogen peroxide (Reagent 2). Radicals produced by the

hydroxyl radical, including the brown dianisidine radical cation, are also potent in biological terms. Thus, it is possible to measure the anti-oxidative capacity of a sample in terms of inhibition of free radical reactions initiated by production of the hydroxyl radical.

### Measurement of Oxidative Stress Index

The OSI was the TOS-to-TAS ratio, but TAS values were changed to mmol/l. Each OSI was calculated as follows: OSI (arbitrary units) = TOS (mmol $H_2O_2$/l)/TAS (mmol Trolox/l) [11].

### Statistical Analysis

The Statistical analysis was carried out using the Statistical Package for the Social Sciences version 16.0 software for Windows (SPSS Inc, Chicago, Illinois, USA). All quantitative variables were estimated using measures of central location (i.e. mean and median) and measures of dispersion (i.e. standard deviation (SD)). Data normality was checked using the Kolmogorov–Smirnov tests of normality.

For the comparison of biochemical values of patients on different days, the Repeated ANOVA test was applied ($p < 0.05$ was accepted as statistically significant). To determine the days between which there were differences, the Bonferroni test was administered as a post hoc test ($p < 0.016$ was accepted as statistically significant). For the comparison of qualified data, Chi squared test was used. Pearson's correlation test was used in order to assess the correlations among data. For the comparison of qualified data and all correlations, $p < 0.05$ was considered to be significant.

## Results

As for the sex distribution of patients, 10 of them were female and 17 male. The average age of patients was 28.4 ± 4.7.

### Total Oxidant Status (TOS)

Even though the post-operative day 1 value (14.2 ± 5.3) was increased as compared to the pre-operative value (11.3 ± 6.3), no significant differences were found in between ($p > 0.05$) (Table 1). The TOS value on post-operative day 15 (23.6 ± 5.9) was significantly higher than the value on post-operative day 1 (14.2 ± 5.3) ($p = 0.011$) (Table 1). The TOS value on post-operative day 15 was significantly higher than the pre-operative value ($p = 0.001$) (Table 1) (Fig. 1).

**Table 1** A comparison of DNA damage and oxidative status parameters

| Parametres | Preop Mean ± Std Dev | Postop 1st day Mean ± Std Dev | Postop 15th day Mean ± Std Dev |
|---|---|---|---|
| **Total anti-oxidant status** *TAS - µmol TroloxEqv/L* | 0.33 ± 0.24 | 0.24 ± 0.20 | 0.14 ± 0.08 |
| | ★ | ★ | |
| **Total oxidant status** *TOS - µmol $H_2O_2$Eqv/L* | 11.3 ± 6.3 | 14.2 ± 5.3 | 23.6 ± 5.9 |
| | + | ★ | |
| **Oxidant status index** **OSI** - *TOS/TASX100* | 0.57 ± 0.12 | 1.12 ± 0.25 | 1.92 ± 0.38 |
| | ★ | ★ | |
| **Mononuclear leukocyte DNA Damage** *(arbitary units)* | 24.6 ± 11.4 | 27.5 ± 13.8 | 24.7 ± 8.7 |
| | + | + | |

★p<0.016 + p>0.016

For the comparison within group, the Repeated ANOVA test was applied ($p < 0.05$ was accepted as statistically significant). To determine the days between which there were differences, the Bonferroni test was administered as a post hoc test ($p < 0.016$ was accepted as statistically significant)

### Total Anti-oxidant Status (TAS)

The post-operative day 1 value (0.24 ± 0.20) was significantly higher than the pre-operative value (0.33 ± 0.24) ($p = 0.008$) (Table 1). The TAS value on post-operative day 15 (0.14 ± 0.08) was significantly higher than the pre-operative day 1 (0.24 ± 0.20 ($p = 0.003$) (Table 1). The TAS value on post-operative day 15 was significantly higher than the pre-operative value ($p = 0.001$) (Table 1) (Fig. 2).

### Oxidant Status Index (OSI)

The post-operative day 1 value (1.12 ± 0.25) was significantly higher than the pre-operative value (0.57 ± 0.12) ($p = 0.012$) (Table 1). The OSI value on post-operative day 15 (1.92 ± 0.38) was significantly higher than the value on post-operative day 1 (1.12 ± 0.25) ($p = 0.015$) (Table 1). The post-operative day 15 OSI value was significantly higher than the pre-operative value ($p = 0.001$) (Table 1) (Fig. 3).

### Mononuclear Leukocyte DNA Damage

Even though the post-operative day 1 value (27.5 ± 13.8) was significantly increased as compared to the pre-operative value (24.6 ± 11.4), no significant differences were found in between ($p > 0.05$) (Table 1). There were no significant differences between the values on post-operative day 15 (24.7 ± 8.7) and post-operative day 1 (27.5 ± 13.8) ($p > 0.05$) (Table 1). There were no significant differences between the post-operative day 15 and pre-operative values ($p > 0.05$) (Table 1) (Fig. 4).

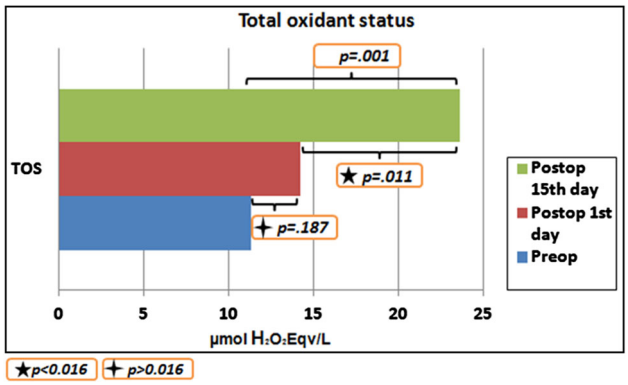


**Fig. 1** Change in total oxidant status over time. For the comparison within group, the Repeated ANOVA test was applied ($p < 0.05$ was accepted as statistically significant). To determine the days between which there were differences, the Bonferroni test was administered as a post hoc test ($p < 0.016$ was accepted as statistically significant)

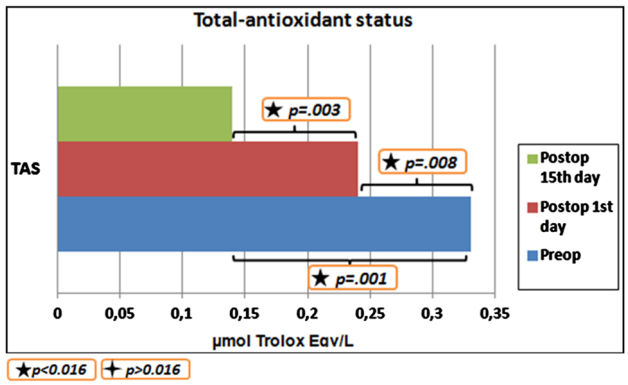


**Fig. 2** Change in total antioxidant status over time. For the comparison within group, the Repeated ANOVA test was applied ($p < 0.05$ was accepted as statistically significant). To determine the days between which there were differences, the Bonferroni test was administered as a post hoc test ($p < 0.016$ was accepted as statistically significant)

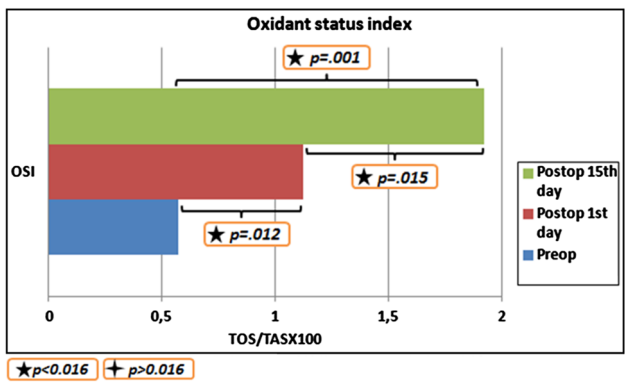


**Fig. 3** Change in oxidative stress index over time. For the comparison within group, the Repeated ANOVA test was applied ($p < 0.05$ was accepted as statistically significant). To determine the days between which there were differences, the Bonferroni test was administered as a post hoc test ($p < 0.016$ was accepted as statistically significant)

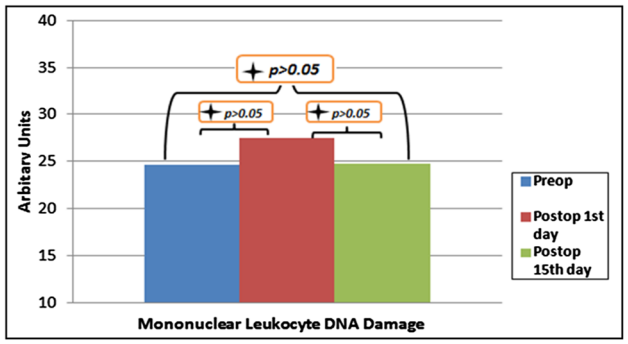


**Fig. 4** Change in DNA damage over time. For the comparison within group, the Repeated ANOVA test was applied ($p < 0.05$ was accepted as statistically significant)

## Correlation Analysis

Postop day 1: There was a negative correlation between TAS values and OSI ($r = 0.457$, $p = 0.017$). There was a significant difference between TOS values and OSI, as well ($r = 0.548$, $p = 0.003$). There was a weak correlation between OSI and DNA damage ($r = 0.397$, $p < 0.05$).

Post-operative day 15: A negative correlation was present between the TAS value and OSI ($r = 0.404$, $p = 0.037$). There was also a negative correlation between TOS values and OSI ($r = 0.554$, $p = 0.003$). There were no correlations between OSI and DNA damage ($r = 0.028$, $p = 0.889$).

## Discussion

The radiofrequency method was used for the first time by Powell et al. [12] in the year 1996 for the volumetric reduction of the tongue in patients with obstructive sleep apnea. It began to be used in various fields as of that date. With respect to the mechanism of action, it does induce ion stimulation in the tissue, increases the local temperature and results in thermal damage in the deep mucosa without harming the surface. It causes sub-mucosal fibrosis in the long term thereby leading to reduced tissue volume without harming the mucociliary activity [13]. Even though electrocautery or laser treatment can create a heat of 800 °C, this temperature is approximately 85 °C with radiofrequency therapy. Therefore, less mucosal damage and necrosis develop [14]. Currently, radiofrequency administration for inferior turbinate hypertrophy is an effective surgical treatment method with proven reliability and few complications, which is conducted even in office conditions.

Oxidative stress (OS) and reactive oxygen species (ROS) development demonstrates the imbalance between antioxidant mechanisms triggered by the organism to detoxify these radicals. In case of oxidative stress, the

antioxidants are used for detoxifying oxidant molecules and this results in a decrease in the antioxidant status [15]. Recent studies demonstrate a close relationship between oxidative stress and DNA damage [5].

There are several methods that used to monitor the gene damage in mononuclear leukocytes as an indicator of the overall damage on genes. These include the micronucleus (MN) test, chromosomal aberration analysis, sister-chromatid exchange (SCE) test, gene mutation tests and comet assay test. Among these tests, the comet assay (single-cell gel electrophoresis) is a simple, fast, sensitive test that is used very frequently for detecting the extensity of endogenous DNA damage with well-demonstrated genotoxicity. This test was used for the first time in our study in order to determine the DNA damage among people who underwent radiofrequency. Furthermore, the parameters of total oxidative status (TOS) and total antioxidant status (TAS) were used to assess the oxidative status in our study. These parameters were used since it was not practical for the individual measurement of various oxidant and antioxidant molecules and it was more significant to determine the total effect [16, 17].

In a study conducted by Vijayalaxmi et al. [4], the venous blood collected from voluntary healthy individuals was exposed to pulsed-wave 2450 MHz radiofrequency radiation for 2 h and it was demonstrated that DNA single-strand breaks and alkali-labile side damage on human lymphocytes were not induced significantly. In our study, the alkaline comet assay method and 2.2 MHz radiofrequency were used to identify the DNA damage. Even though the DNA damage in the samples taken on post-treatment day 1 was higher than the pre-treatment values, the difference between them was not statistically significant (Fig. 4). There were no differences between the damage in samples collected on post-treatment day 15 and the 1 before the treatment (Fig. 4). Based on this result, it can be suggested that radiofrequency treatment may cause inflammatory stress and the related oxidative stress resulted in a slightly systemic DNA damage in the acute phase; however, this damage had been repaired on day 15 via DNA repair mechanisms.

Intranasal phototherapy is also an effective and reliable method that is used for similar indications. Koreck et al. [18] conducted a study in which they investigated the efficacy of phototherapy on allergic rhinitis as well as the effects of UV light on oxidative stress and DNA damage in nasal epithelial cells. In this study, they showed that inflammatory stress and the related oxidative stress resulted in a significant increase in DNA damage among patients with symptomatic allergic rhinitis studied by the Comet assay method. Similarly, the same study also identified a slight increase in the DNA damage of samples collected immediately after the last phototherapy session, however, a significant decrease was seen in the DNA damage in samples collected on day 10. It was concluded that the DNA repair mechanisms was activated in this 10-day period. In our study, there was a slight increase in DNA damage on day 1 as compared to pre-treatment samples. For the samples collected on day 15 after the treatment, there were no significant differences in terms of the DNA damage as compared the status before the treatment and on day 1 after treatment. This slight increase on day 1 may be due to the significant increase in the Oxidant Status Index (OSI) values. Because a positive correlation was identified between OSI and DNA damage. As for the total anti-oxidant status (TAS) values, they were observed to have decreased at a statistically significant level on days 1 and 15. Based on this result, it is concluded that the local administration of radiofrequency on the inferior turbinate increases the total oxidant status, thereby causing a temporary systemic DNA damage; however, it is immediately repaired by the repair mechanisms.

## Conclusion

Even though the radiofrequency treatment is a local treatment, it causes an increase in oxidative stress systematically, hence an increase in DNA damage, albeit statistically insignificant, due to the direct effect of radiofrequency in the acute phase. For that reason, the administration of radiofrequency on inferior turbinate should be used only on indicated patients rather than being part of routine surgery. In vitro and in vivo studies are required in order to learn the long-term results of radiofrequency treatment.

### Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical Standard** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed Consent** Informed consent was obtained from all individual participants included in the study.

## References

1. Lanier B (2007) Allergic rhinitis: selective comparisons of the pharmaceutical options for management. Allergy Asthma Proc 28:16–19
2. Utley DS, Goode RL, Hakim I (1999) Radiofrequency energy tissue ablation for the treatment of nasal obstruction secondary to turbinate hypertrophy. Laryngoscope 109:683–686

3. Lin HC, Lin PW, Friedman M, Chang HW, Su YY, Chen YJ et al (2010) Long-term results of radiofrequency turbinoplasty for allergic rhinitis refractory to medical therapy. Arch Otolaryngol Head Neck Surg 136:892–895. doi:10.1001/archoto.2010.135
4. Vijayalaxmi, Leal BZ, Szilagyi M, Prihoda TJ, Meltz ML (2000) Primary DNA damage in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation. Radiat Res 153:479–486
5. Hilali N, Kocyigit A, Demir M, Camuzcuoglu A, Incebiyik A, Camuzcuoglu H et al (2013) DNA damage and oxidative stress in patients with mild preeclampsia and offspring. Eur J Obstet Gynecol Reprod Biol 170:377–380. doi:10.1016/j.ejogrb.2013.07.031
6. Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191
7. Kobayashi H, Sugiyama C, Morikawa Y et al (1995) Comparison between manual and microscopic analysis and computerized image analysis in single cell electrophoresis assay. MMS Commun 3:103–115
8. Kocyigit A, Keles H, Selek S, Guzel S, Celik H, Erel O (2005) Increased DNA damage and oxidative stress in patients with cutaneous leishmaniasis. Mutat Res 585:71–78
9. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103–1111
10. Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37:277–285
11. Hilali N, Vural M, Camuzcuoglu H, Camuzcuoglu A, Aksoy N (2013) Increased prolidase activity and oxidative stress in PCOS. Clin Endocrinol 79:105–110
12. Powell NB, Riley RW, Troell RJ, Blumen MB, Guilleminault C (1997) Radiofrequency volumetric reduction of the tongue. A porcine pilot study for the treatment of obstructive sleep apnea syndrome. Chest 111:1348–1355
13. Sargon MF, Celik HH, Uslu SS, Yücel OT, Denk CC, Ceylan A (2009) Histopathological examination of the effects of radiofrequency treatment on mucosa in patients with inferior nasal concha hypertrophy. Eur Arch Otorhinolaryngol 266:231–235
14. Harrill WC, Pillsbury HC, Mc Guirt WF, Stewart MG (2007) Radiofrequency turbinate reduction: a nose evaluation. Laryngoscope 117:1912–1919
15. Young IS, Woodside JV (2001) Antioxidants in health and disease. J Clin Pathol 54:176–186
16. Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37:277–285
17. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103–1111
18. Koreck A, Szechenyi A, Morocz M, Cimpean A, Bella Z, Garaczi E et al (2007) Effects of intranasal phototherapy on nasal mucosa in patients with allergic rhinitis. J Photochem Photobiol B 89:163–169