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Histologic evaluation of nasal epithelium of the middle turbinate in untreated OSAS patients and during nCPAP therapy*

Sophia Schrödter[1], Eckhardt Biermann[2], Zdenek Halata[1]

[1] ENT-Department, St. Georgs Hospital, Lohmühlenstr. 5, 20099 Hamburg, Germany
[2] Department of Functional Anatomy, University of Hamburg, Martinistr. 52, 20246 Hamburg, Germany

SUMMARY

OSAS-patients complain about nasal disorders. Irritation of the nasal mucosa often leads to termination of nCPAP treatment. The aim of this study is to evaluate whether symptoms are related to histologic changes of the nasal mucosa of the head of the middle turbinate in OSAS-patients.

Semi-thin sections of epon-embedded middle turbinate biopsy samples from 35 male patients (age 51-75 yr) with OSAS were compared with those of 10 healthy men (age 51-75 yr).

In untreated OSAS-patients atrophic epithelium is common whereas ciliated epithelial types are rare. After short-time nCPAP therapy (mean 581h) patches of ciliated epithelium and squamous metaplasia reappear. Short-term nCPAP leads to a partially restoration of the mucosal architecture. During long-time nCPAP therapy (mean 6.737h) squamous metaplasia with conspicuous intraepithelial connective tissue papillae predominates whereas pseudostratified ciliated epithelium is missing. Dense round cell infiltrates in the lamina propria are frequently found.

Rhinitic symptoms in OSAS-patients are correlated with marked histological changes of the respiratory epithelium of the head of the middle turbinate. Histological changes in untreated OSAS differ from those of patients during nCPAP-treatment.

Key words: nCPAP-therapy, sleep apnoea, nasal mucosa, histology, middle turbinate

INTRODUCTION

The obstructive sleep apnea syndrome (OSAS) characterized by repeated episodes of partial or complete upper airway obstruction during sleep is a common condition of the adult middle-aged population more frequently found in men than in women (Young et al., 1993; Duran et al., 2001). The frequency of OSAS increases with age (Duran et al., 2001). OSAS rank among the major public health threats in industrial nations.

Nasal continuous positive airway pressure (nCPAP) therapy introduced by Sullivan et al. in 1981 has been shown to effectively eliminate apneas and hypopneas, improve daytime function and quality of life and therefore is the treatment of choice at least for moderate-to-severe obstructive sleep apnea syndrome (Engleman et al., 1998; D'Ambrosio et al., 1999). Despite benefits compliance with nCPAP varies due to individual tolerance and perception of side effects which have been categorized into nasopharyngeal symptoms, mask discomfort, problems in connection with nCPAP devices and social problems (Brander et al., 1999; Kalan et al., 1999; Lojander et al., 1999; McArdle et al., 1999; Verse et al., 1999; Hui et al., 2001). Long-term compliance is about 60 to 70% (McArdle et al., 1999).

Whereas some nasal complaints of untreated OSAS may disappear during nCPAP therapy sneezing and rhinorrhoea increase significantly (Brander et al., 1999; Lojander et al., 1999; Verse et al., 1999). Nasal side effects of nCPAP therapy (Brander et al., 1999; Lojander et al., 1999; Verse et al., 1999) have been related to mouth leaks causing unidirectional nasal airflows (Hayes et al., 1995) and high flow rates over long periods (Kalan et al., 1999). Successive changes in the balance of benefits to side effects during long-term nCPAP treatment may lead to termination of nCPAP treatment even after years (McArdle et al., 1999). There are only two papers dealing with ultrastructural changes of the nasal epithelium under nCPAP therapy with only 10 patients and a maximum of one year of treatment (Constantinidis et al., 2000a, Constantinidis et al., 2000b). A preliminary study revealed that age-related changes

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of the nasal mucosa have to be considered (Schrödter et al., 2003).
We will show whether nCPAP can be related to histological or ultrastructural changes of the nasal respiratory mucosa and whether these are characteristic for nCPAP-treatment.
Limitations of this cross-sectional study are based on the study design. A long-term follow-up study design would eliminate the variability between subjects. But 10 years follow-up accompanied by repeated biopsy sampling do not seem realizable.

MATERIAL AND METHODS

Biopsy sampling

Biopsy specimens were collected (by E.B.) from 35 male patients (age 51-75 yr.) with OSAS: 10 untreated OSAS patients (61.1 ± 7.1 yr.), 12 OSAS patients (60.5 ± 5.9 yr.) using their nCPAP devices for less than 2.000h (581 ± 370 h) and 13 OSAS patients (62 ± 5.9 yr.) using their devices for more than 2.000h (6737 ± 3475h). Time on nCPAP was measured by a built-in time counter. Patients were not medicated for nasal symptoms. Patients prescribed on drugs were excluded if side effects of the medication were supposed to affect the functions of the nasal mucosa. All patients had to fulfil the following requirements: no chemo- or radiotherapy, no allergic rhinitis, no affection of lung or bronchia, no marked septum deviation, no diabetes, no heavy smoking, no alcoholics, no psychopharmacological therapy.
For reference 10 healthy men (age 51-75 yr., 57.5 ± 3.7 yr.) being treated at the ENT-department of St. Georgs hospital (Hamburg) for complaints not related with the nose were chosen. The control group had to fulfil the following requirements: no OSAS, no operations, no chemo- or radiotherapy, no allergic rhinitis, no affection of the respiratory tract for at least 3 months, no marked septum deviation, no diabetes, no heavy smoking, and no drugs with possible side effects on nasal mucosa for at least 3 months. All 45 biopsy specimens were chosen out of a larger sample collection. All specimens which fulfilled the collective requirements (age, sex, patients collective) were evaluated.
Informed consent was obtained in all cases according to ethical committee stipulations.

Biopsy samples were obtained from the head of the middle turbinate (inaccessible with finger) under local or general anaesthesia using a sharp cupped forceps (3mm) and immediately fixed in 6% glutaraldehyde in 0.05% phosphate buffer. After postfixation with 1% OsO_4 (0,1 M phosphate buffer and 1% saccharose) specimens were embedded in Epon 812 (Luft, 1961). Ultrathin sections were contrasted with uranylacetate / lead citrate and viewed with a Philips 300 TEM.

Evaluation of histologic sections

Sections perpendicular to the epithelial surface of the more or less spherical specimens were evaluated. Histological analysis was double-blinded. Six epithelial types were discriminated: pseudostratified ciliated, stratified ciliated, cuboidal, squamous metaplasia, atrophic and disintegrating. Four epithelia not fitting these categories were listed as 'other'. For characterisation of epithelial types see Schrödter et al. (2003). Most specimens show more than one epithelial type, quantitative analysis was impossible.
Basement membrane thickness was measured in semi-thin sections. Mean values for 10 randomly chosen points for each epithelial type and specimen were calculated.

RESULTS

Histology of the epithelium

Comparing OSAS patients with healthy men, a marked decrease in ciliated epithelial types was found (Figure 1). 50% of biopsy specimens of the control group show ciliated epithelium (pseudostratified or stratified) whereas in the untreated OSAS patients ciliated epithelium was found in only 20% of the samples. Atrophic epithelium was predominant in untreated OSAS patients and found in 60% of the biopsy specimens.
In patients during short-term nCPAP treatment (581 ± 370 h) the higher frequencies of ciliated epithelial types (50% of samples) tend to show epithelial recovery. Atrophic epithelia are decreased from 60% to 41.7% of patients. Squamous metaplasia is markedly increased (10% of untreated OSAS patients to 50% of short-term nCPAP patients).

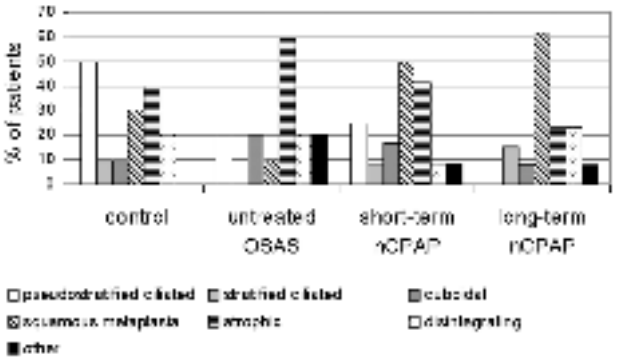

Figure 1. Composition of the epithelium of the head of the middle turbinate in biopsy samples of 10 healthy men (control), 10 patients with untreated OSAS, 12 short-term nCPAP patients and 13 long-term nCPAP patients. Due to the patchy arrangement of different epithelial types in most biopsy samples (most samples show at least two different epithelial types) the addition of percentages in each subgroup can exceed 100%.

After long-term nCPAP treatment (6737 ± 3475 h) pseudostratified ciliated epithelium is missing. Stratified ciliated epithelium is rarely found (15.4% of patients). The predominant epithelial cover is squamous epithelium (Figure 2), which is observed in 61.5% of the samples. The frequency of atrophic epithelium is further decreased compared to short-term treatment (41.7% in short-term to 23.1% in long-term treatment patients).

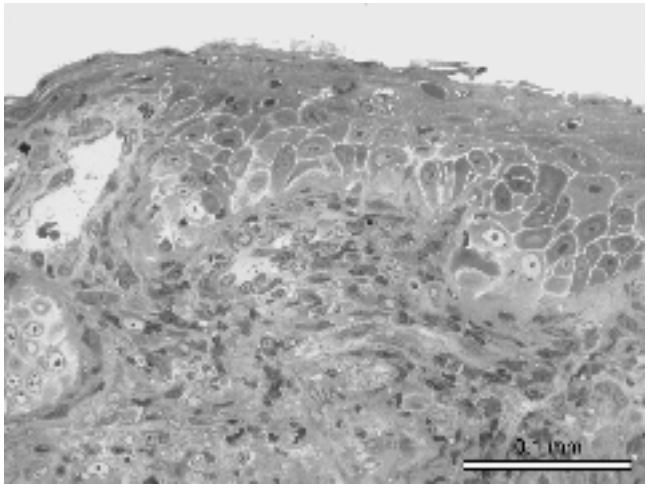

Figure 2. Squamous metaplastic epithelium of the head of the middle turbinate in OSAS patient after 6.430h of nCPAP treatment.

Neither goblet cell hyperplasia nor hyperplasia of basal cells showed definite correlation to one of the patients groups or controls. No specific ultrastructural changes could be found in the epithelial cells. Marked widening of intercellular spaces (Figure 2) could be observed in some specimens but no certain correlation with one of the collectives was detectable.

Connective tissue papillae in the epithelium

Connective tissue papillae with one or more capillary loops are frequently found in multilayered epithelial types such as cuboidal and squamous epithelia. The high frequencies of connective tissue papillae found in patients during nCPAP treatment are correlated to the predominance of these epithelial types. Contrarily one would expect few papillae in untreated OSAS patients. The astonishingly high frequency of papillae in these samples are mostly found in disintegrating epithelia (Figure 3) including samples showing detached parts of the epithelium including papillae with capillaries. In some samples atrophic epithelia were found including remains of connective tissue papillae.

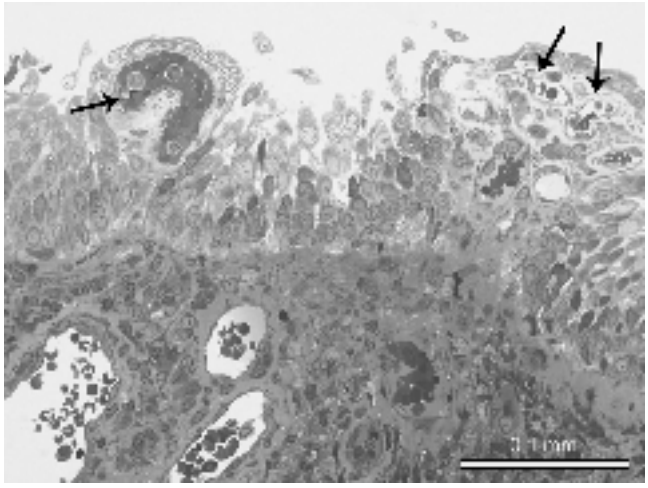

Figure 3. Disintegrating epithelium of the head of the middle turbinate in untreated OSAS patient. Parts of the epithelium including connective tissue papillae with capillary loops are detached. Arrows = capillary loops.

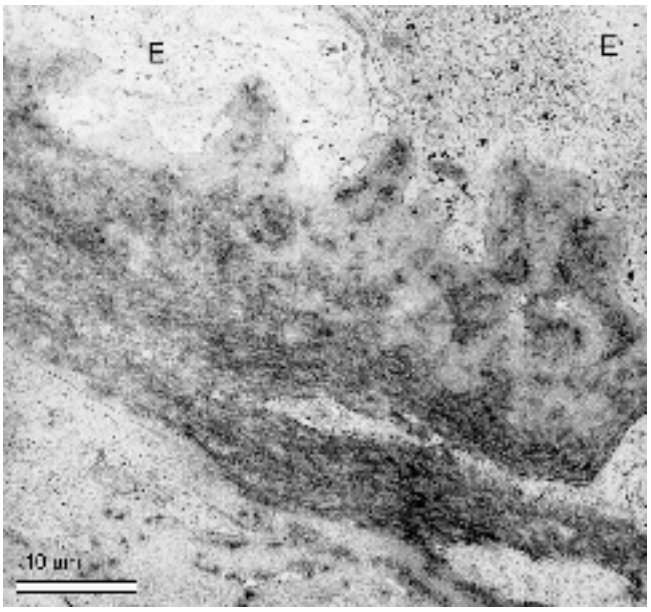

Figure 4. Basement membrane in TEM preparation of biopsy sample of OSAS patient after 13.300h of nCPAP treatment. A basal lamina (lamina densa) is not detectable. E = basal cell of squamous metaplastic epithelium.

Basement membrane

Mean basement membrane thickness was nearly the same in all patient groups and in the controls. Conspicuously thickened basement membranes were found underlying atrophic and disintegrating epithelial types whereas those underlying squamous metaplasia were thin.

In some patients of the long-term nCPAP-group basement membranes show hyaline appearance in TEM-preparations (Figure 4).

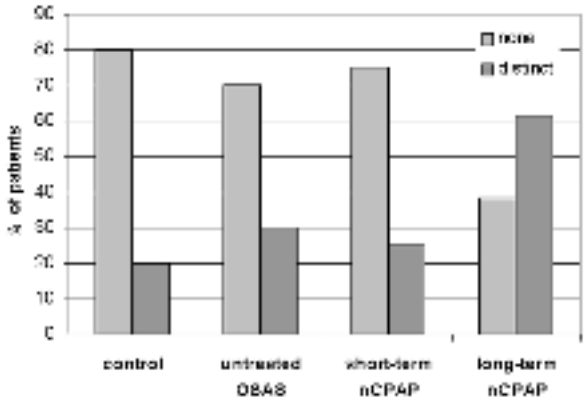

Figure 5. Round cell infiltrate in biopsy samples of 10 healthy men (control), 10 untreated OSAS patients, 12 short-term nCPAP patients and 13 long-term nCPAP patients. Long-term nCPAP patients show a remarkably high incidence of round cell infiltrate.

Round cell infiltrate in the lamina propria

Infiltration of round cells in the lamina propria is common in nasal mucosa. In untreated OSAS patients and patients during short-time nCPAP treatment the occurrence of round cell infiltrates do not differ from healthy individuals (Figure 5). But long-term nCPAP treatment provokes a conspicuously elevated frequency of round cell infiltrates. Round cell infiltrates were shown in 61.5% of patients during nCPAP treatment. Round cell infiltrates comprise lymphocytes, monocytes and granulocytes.

DISCUSSION

Histology of the epithelium and nasal symptoms in untreated OSAS patients

As Lojander et al. (1999), Brander et al. (1999) and Massie et al. (1999) could show patients suffering from OSAS complain about discomfort of the inner nose before nCPAP treatment is started. It can be shown that other than e.g. in allergic rhinitis where no changes of the epithelium can be observed in histological sections discomfort in OSAS is correlated with histological changes of the nasal mucosa. Common symptoms of OSAS patients are stuffiness of the nose, dryness of the nose and throat, sneezing and rhinorrhoea (Brander et al., 1999; Lojander et al., 1999; Massie et al., 1999). Regression of ciliated epithelial types and the frequent prevalence of atrophic epithelia in untreated OSAS may be related to changes in gas circulation during abnormal respiration. Unidirectional airstreams caused by snoring (open mouth) impede recovery of humidity and temperature in the anterior nasal segment which is very effective (while not completely understood) during normal breathing (Keck et al., 2001). Changes of the "physiologic climate" lead to drying of the mucus blanket and crusting. Humidification of the inspired air is dependent on the mucus blanket acting as an interface between the delicate respiratory epithelium and the air stream which is continuously supplemented by subepithelial glands, by transudate from subepithelial capillaries, by epithelial goblet cells and secretions, and by cell debris from mucosal cells (Lucas et al., 1934). While the origin of the secretions is known, neither the contributions of the secreting elements nor the control of these elements are well understood and even our understanding of the diverse facets of the two-layer mucus blanket is still fragmentary (Taylor, 1974). Further studies will have to show whether at least partial loss of the continuous functional mucus blanket, impaired mucociliary clearance and water holding capacity are cause or consequence of histological changes in the nasal epithelium in OSAS-patients.

Nasal symptoms during nCPAP therapy

The most common nasal side effects of nCPAP therapy without humidification of the inspired air are dryness of the nasal mucosa, nasal congestion or stuffiness, crusting, rhinorrhoea and sneezing (Brander et al., 1999; Hayes et al., 1999; Lojander et al., 1999; Pepin et al., 1999; Verse et al., 1999).

Though nasal complaints in untreated OSAS patients and after application of nCPAP differ only concerning frequency and severity of symptoms (Brander et al., 1999) we found remarkable differences in epithelial histology. Patchy loss of ciliated epithelial types interferes with the normal mucociliary transport. The remarkable increase in epithelial transformation to squamous metaplasia in our samples can be related to accumulation of traumatic effects due to high flow rates and unidirectional air flow with drying and cooling of the epithelium caused by inescapable mouth leaks during nCPAP (Martins-De-Araujo et al., 2000). Squamous epithelium with high connective tissue papillae is characteristic for the nasal valve area (Eccles, 1995), where under normal conditions the highest flow rates can be measured (Kelly et al., 2000).

The nasal epithelium seems to partly improve under short-time nCPAP. One possible explanation might be found in the higher humidity and temperature of inspired air even without humidifier (humidity of air in the mask and upper tube are increased, unpublished results).

Connective tissue papillae are frequently found in squamous epithelia which are predominant in biopsy samples of persons during nCPAP treatment. Predominance of squamous metaplasia can be discussed as protection shield against traumatic stimuli and drying and cooling of the mucosa. The mechanical load is especially elevated by high inspiratory flows during nCPAP (Kesten et al., 1990). Connective tissue papillae with dense capillary loops may be responsible for the storage and delivery of heat and water for the more difficult conditioning of the inspired air during nCPAP.

NCPAP treatment without heated humidification induces an inflammatory response of the respiratory epithelium (Constantinidis et al., 2000a, 2000b) which apparently becomes chronic after long-time application recognizable by predominance of round cell infiltrates – as releasing factors pressure stimuli and increased air flow with drying and cooling of the epithelium are discussed (Naclerio et al., 1995).

Hyaline basement membranes found in some long-term nCPAP preparations may be dependent on changes in basement membrane composition which needs further investigation.

Whereas Lojander et al. (1999) doubted whether humidification improves nCPAP treatment success, Massie et al. (1999) found adverse side effects and symptoms improved and duration of usage prolonged after application of heated humidification during short-time nCPAP treatment (8 weeks). Wiest et al. (2001) could show that modern humidification systems improved relative and absolute humidity even under changing room temperature and humidity regimes with a minimum of 19.1g/m^3 humidity performance which is only slightly less than 23g/m^3 humidity performance requested for intubated or tracheotomized patients. Further studies will have to show whether heated humidification leads to improved long-time compliance and whether it can prevent or slow down epithelial

transformation or improve respiratory epithelium histology during long-term nCPAP-treatment. Follow-up study design would be desirable.

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Sophia Schrödter
Department of Functional Anatomy
University of Hamburg
Martinistr. 52
20246 Hamburg
Germany

Tel.: +49-040-42803-2578
Fax: +49-040-42803-2845
E-mail: schroedt@uke.uni-hamburg.de