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Nasal Mucosal Morphology after Altera tion of Air Stream

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Abstract

Introduction The nocturnal application of nasal continuous positive airway pressure is used for treatment of sleep apnoea. The study of the histopathological changes that accompany its application is needed for proper evaluation.

Subjects and Methods: Fifteen adult rabbits were used in this study, 14 were the subject of the experiments and one animal used as control. Under combined general and local anaesthesia, ring of skin and epithelium was removed from the left nostril, the margins of the wound were undermined, averted and brought together with mattress sutures, effectively closing the nostril. The animals were sacrified at intervals. The duration of surgical closure of one nostril was four days in 7 animals and three months in 7 others. Strip of bony and cartilaginous septum was removed after marking the closed side with a silk stitch. In each interval 4 animals were used for transmission electron microscope (TEM) and 3 for scanning electron microscope (SEM).

Results: On the side with sealed nostril the number of goblet cells increased, while the number of ciliated cells decreased. In contrast, on the open side of the nose disappearance of the ciliated cells and a transformation of the respiratory epithelium into thickened multilayered squamous epithelium with no goblet cells.

Introduction

The nocturnal application of nasal continuous positive airway pressure (nCPAP) was first described in 1981 by Sullivan *et al*. Then in 2003 by Lang *et al.* and recommended for treatment of sleep apnoea. Since then n CPAP has established itself in the therapeutic management of moderate and severe obstructive sleep apnoea syndrome (Hoffstein *et al*., 1992).

Room air is supplied with a positive pressure to the nose through a nasal mask by means of a blower unit, thus leading to a pneumatic stabilization of the upper respiratory airways. The apnoea phases are thus eliminated, snoring is distinctly reduced and subjective patient complaints, such as daytime somnolence and lack of concentration, are largely overcome (Ripberger & Pirsig, 1994 and Kelly *et al.*, 2000).

Only continuous therapy is able to prevent severe consecutive cardiopulmonary disorders and to lower mortality rate (He *et al*., 1988 and Partinen *et al*., 1988).

Long-term acceptance of the n CPAP-masks by patients ranges from 67.4% to 89.7% (Partinen *et al*., 1988) when nCPAP-therapy is discontinued, the patients' complaints return.

Long-term therapy can; however, be a burden for the patient if various complications develop. Apart from problems related to mask usage (e.g. pressure spots, skin reaction, claustrophobia, and problems with traveling) there have been reports of eye irritation, noise disturbance caused by the nCPAP-blower, headache and tinnitus (Katsantonis *et al.*,1988). The most frequent and significant side-effect of nCPAP-therapy, with a reported frequency of 20-70%, is irritation of the upper respiratory pathways and in particular of the nasal mucosa (Kuhl *et al.*, 1997). Changes in the mucosa that are causative for the entire spectrum of symptoms are induced by applying cold and dry room air under elevated pressure. Typical complaints that can develop over period of a few days include nasal dryness with accompanying rhinitis, rhinopharyngitis, laryngitis, sinusitis, hypertrophy of the nasal conchae, nasal bleeding and an increased rate of infection of the upper airway (Mayer-Brix *et al*., 1989 and Boyce & Eccles, 2006). In infections, products of bacteria and mediators released from mucosa have an impact on mucociliary function (Konietzko, 1986). So that mucociliary transport is impaired in patients with recurrent or chronic respiratory infection (Nuutinen *et al*., 1983; Toremalm, 1984 and Majima *et al*., 1986). Morphological changes of respiratory mucosa are one important factor. Loss of cilia and ciliated cells, ultrastructural changes of cilia and changes in the composition of the respiratory mucus in various infections have been reported (Reimer *et al*., 1978 and Afzelius, 1981). Alteration of nasal airflow induces a functional transformation of the mucosa, the extent of which varies and can range from ciliated epithelium with partial loss of cilia, via columnar epithelium without cilia, to squamous epithelium (Camara *et al*., 2006).

Subjects and Methods:

This study was carried out in ENT, Anatomy and Clinical Pathology departments at Al-Azhar and Ain Shams Universities in the period from 1/2005 to 1/2006.

Fifteen adult rabbits were used in this study; 14 were the subject of the experiments and one animal used as control. Under combined general and local anaesthesia, a ring of skin and epithelium was removed from the left nostril, the margins of the wound were undermined, averted and brought together with mattress sutures. Seven of the closed nostril animals were sacrified after four days and the rest were sacrified after 3 months. A strip of bony and cartilaginous septum was removed after marking the closed side with a silk stitch. Specimens from 4 animals of each group were used for TEM; specimens of other 3 animals were used for SEM. For TEM, specimens were cut into small pieces and fixed by immersion in 2.5% glutaraldehyde for 2 hours at 4^{o}C. The tissues were postfixed in 1% osmium tetroxide solution for 90 min at 4^{o}C dehydrated in an ascending grades of ethanol and embedded in epon. Semithin sections were cut at 1 µ m with an LKB 8800 ultramicrotome and stained with toluidine blue. Ultrathin sections were contrasted with uranylacetate and lead citrate and examined with electron microscope. For SEM; specimens were processed according to Malick *et al*. (1976) and Lee *et al*. (2005). Mucosal samples were washed in saline and fixed by immersion in gluteraldhyde for 24 hours, dehydrated by graded series of alcohol, critical point dried with liquid carbon dioxide, coated with gold and examined with SEM. The ultrastructural features were recorded and compared.

Results

Control animal: The mucous membrane of the septum of the animal not subjected to closure of one nostril showed the expected pseudo-stratified columnar, ciliated epithelium with a mixture of goblet and ciliated cells (Fig. 1). The epithelium varied considerably between adjacent areas in thickness, density of ciliated cells, and surface regularity. Rarely new cilia were observed in the process of formation of basal bodies in the upper portion of the cell (Fig. 2). Another rare finding was that of club-like shaped cilia "homogenous cilia" (Fig. 3).

Experimental animals: Nearby areas varied extremely in each of the specimens examined. So marked was this variation that no consistent differences could be established between the short-term (four-day) and the chronic (three months) specimens.

Findings on the surgically closed side of the nose: presented unevenness of the surface after deprivation of airflow. It seemed as the ciliated surface was being thrown into folds because of the exuberance of the numerous swollen goblet cells. Ciliogenesis (formation of new cilia) was active on the closed side. Basal bodies were found in the upper portion of cells topped by microvilli (Fig.4). The cytoplasm of these cells tended to be quite dark, with numerous ribosomes.

Another effect of airway closure on the closed side was the increased number of "homogenous cilia". These are cilia that have a club-like appearance. A few of these were sometimes found among normal-looking, mature cilia (Fig. 5).

On the open side of the nose ciliated cells and cilia disappeared in the basal side (Fig. 6) and can range from ciliated epithelium with partial loss of cilia (Fig. 7), via columnar epithelium without cilia to squamous epithelium (Fig.8) without goblet cells. The cell nuclei became irregular and contain varying number of nucleoli (Fig. 7).

Fig. (1): Transmission electron micrograph of epithelium of nasal septum from control rabbit, showing pseudostratified columnar cells (C) with goblet cells (G) and ciliated surface (Ci). Note basal bodies (BB) appeared in the upper part of cells. (X 8000).

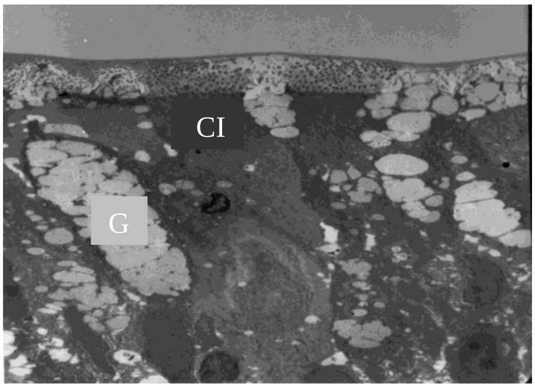

Fig. (2): Transmission electron micrograph of epithelium of nasal septum from control rabbit, showing normal epithelial dark cells (D), goblet cells (G). Note surface of cells covered by cilia (Ci) and basal bodies (BB)appeared in the upper part of cells. (X 10000)

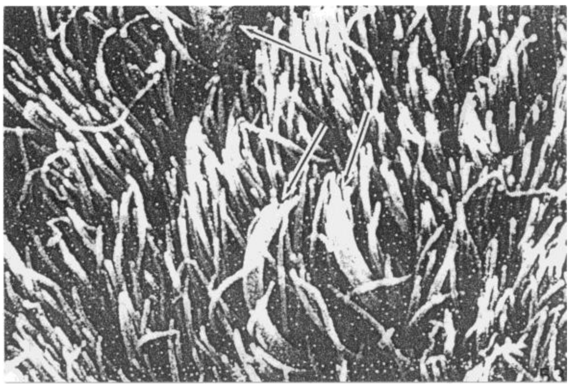

Fig. (3): scanning electron micrograph of epithelial surface of control rabbit, showing several homogenous cilia (arrows) (X 9000)

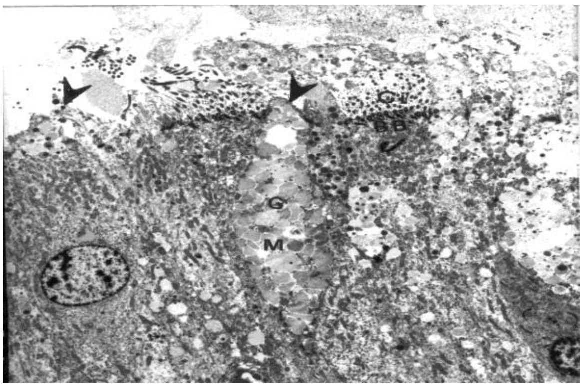

Fig. (4): Transmission EM of respiratory epithelium of nose, showing increased number of goblet cells (G) containing mucus granules (M) many of them are seen discharging their content on the surface. The epithelial surface is uneven showing infoldings by the swollen goblet cells (arrows). The cilia are diminished (Ci), but ciliogenesis is noted by basal bodies in the upper portion of the cells (BB) (X 8000).

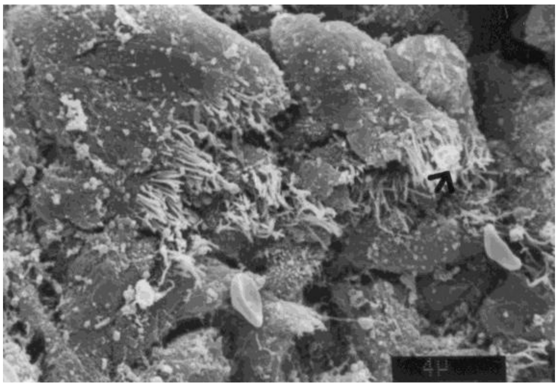

Fig. (5): Scanning electron micrograph of epithelium of nasal septum of rabbit after surgical closure of this side of the nose for 3 months, showing several homogenous cilia (arrow) (X 8000)

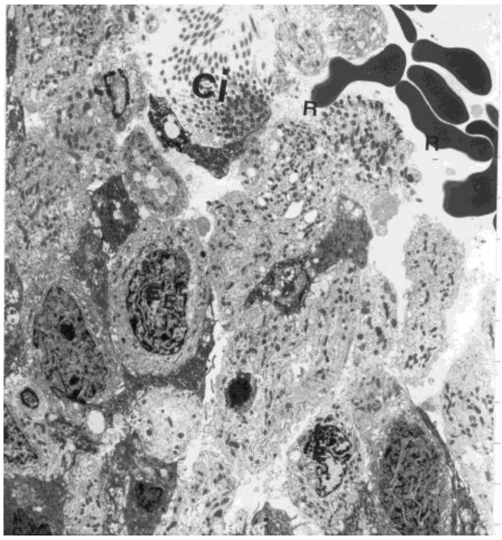

Fig. (6): Transmission EM of nasal epithelium from opened side of the nose showing epithelial multilayered (E), ciliated surface (Ci) with infoldings, RBCs in lumen (R). No goblet cells are seen. (X 10000)

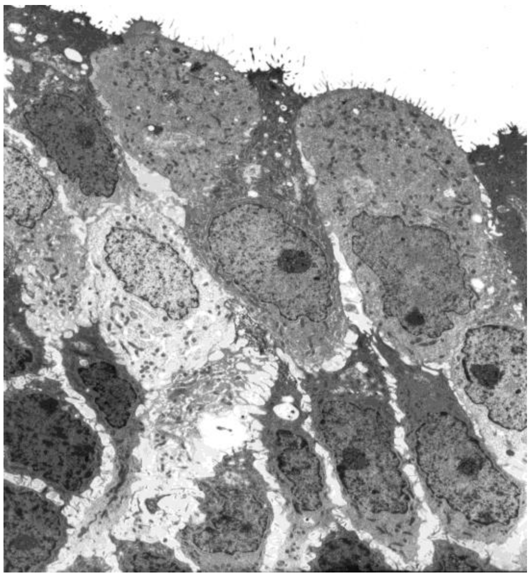

Fig. (7): Transmission EM of nasal epithelium showing multilayered cliated epithelium with partial loss of cilia (X 8000)

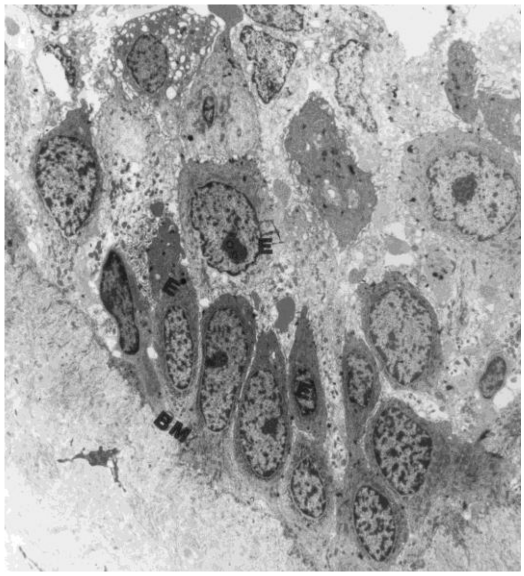

Fig. (8): Transmission EM of epithelium from opened side of the nose showing squamous metaplasia (E) loss of goblet cells and loss of cilia basement membrane (BM) (TEM X 8000)

Discussion

The effect of nasal airflow on the structure and function of the nasal mucosa has been known for many years. Alteration of nasal airflow induces a functional transformation of the mucosa, the extent of which varies and can range from ciliated epithelium with partial loss of cilia, via columnar epithelium without cilia, to squamous epithelium (Jahnke, 1972 and Shin & Heo, 2005)). The resulting pathophysiological alterations of the mucosa give rise to a variety of rhinological clinical manifestations.

In the present study, sealing the nostril resulted in an increase in the number of goblet cells, while the number of ciliated cells decreased. In contrast, on the open side of the nose, where the air stream had increased due to closure of the contralateral side, the ciliated cells disappeared and the respiratory epithelium was transfomred into a thickened, multilayered squamous epithelium with no goblet cells.

In his pioneer experimental work on rabbits Hilding and Hilding (1970) also demonstrated that the epithelium of the nasal mucosa is transformed into a thick stratified squamous epithelium on the open side of the nose through which the airflow is doubled as compared with normal. On the surgically closed side he also found 3 weeks after the closure, a considerable increase in goblet cells and reduction of ciliated cells. Hilding and Hilding (1970) found pronounced ciliogenesis on the closed side. They concluded that the epithelial types found in the upper respiratory tract are not constant but change under altered external condition.

Tos and Mogensen (1979) reported that after 90 days the epithelium of the closed side was thickened due to hyperplasia of basal cells but it gradually returned to a normal thinner, regular epithelium with increased number of goblet cells starts from the fourth day. However, in our experiment the epithelium did not reach the normal structure. On the patent side, where the airflow was doubled, they found that the cilia were damaged as found in our experiment.

Constantiidis (2000) used nCPAP mask instead of closure nostril and found clumping of microvilli and cilia and modification of epithelial cells with prolongation of mucociliary clearance time due to loss of cilia as examined by a saccharine test before and after treatment.

Similar to experimental alteration of nasal airflow is nCPAP-therapy, the acutely developing rhinitis can take a chronic course and can ultimately induce severe alterations of the nasal mucosa, resulting in a disorientation and destruction of cilia as well as epithelial metaplasia (Ohashi & Nakai, 1983 and Eccles, 2000).

The morphological changes of the nasal epithelium correlate with the functional capacity of the nose and with mucociliary clearance (Toskala *et al.*, 1995).

Boyce and Eccles (2006) found that the continuously elevated nasal airflow influences mucociliary clearance, which in turn is dependent on both ciliary activity and secretory capacity.

The effect of increased airflow and mechanical stimuli can lead to the distruction of cilia and consequently to mucostasis with the corresponding clinical symptoms (Ohashi and Nakai, 1983).

Unilateral occlusion of the nasal vestibule leads to a prolongation of muc-ociliary clearance on the occluded side of the nose. In contrast, on the non-occluded side, mucociliary clearance is distinctly shortened (Deitmer and Erwing, 1986). If airflow through the nose is completely interrupted on both sides, as, for example, in laryngectomized patients with a tracheostoma, mucociliary clearance of the nose is accelerated (Sakakura *et al.*, 1983 and Constantiidis *et al*., 2000).

Fisher *et al*. (1992) comcluded that, mean airflow changes have effects on nasal mucosal structure and function, and our studies suggest that this is a dynamic process which takes time. This is of relevance to the postoperative assessment of patients undergoing surgery to alter the nasal airway.

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التغير التركيبى لمخاطية الأنف بعد تغيير شدة تيار الهواء المار

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تستخدم طريقة زيادة شدة تيار الهواء الليلى لحالات التوقف التنفسى أثناء النوم ويتطلب معرفة تأثير هذة الطريقة على المدى البعيد دراسة التغيرات التى تطرأ على مخاطية الأنف لذلك تمت هذة الدراسة بغرض بحث التغيرات النسيجية للغشاء المخاطى الأنفى المصاحبة لتغير شدة تيار الهواء الداخل إلى التجويف الأنفى كما هو حادث فى التوقف التنفسى أثناء النوم (البهر) حيث يتم العلاج بزيادة شدة التيار الهوائى المار بالتجويف الأنفى.

تمت هذة الدراسة على أربعة عشر أرنباً بالغاً كعينة تجريبية وواحد كعينة ضابطة للتجربة وتم ذلك تحت التخدير العام والموضعى فى مكان الفحص النسيجى حيث تمت إزالة شريط دائرى حلقى من الغشاء المخاطى للجزء الأيسر من التجويف الأنفى ثم خياطة طرفى الشق الجراحى لغلق التجويف الأنفى.

تم ذبح الأرانب على فترتين لفحصها وكانت مدة الغلق الجراحى لإحدى فتحتى الأنف أربعة ايام فى سبعة أرانب وثلاثة شهور فى السبعة الأخرى.

تم الحصول على شريط من الغضروف والحاجز الأنفى من المجموعات التجريبية بعد الذبح مباشرة وأخذت العينات من كل جانب على حدة حيث تم دراسة عينات أربعة أرانب بالميكروسكوب الإلكترونى النافذ وثلاثة بالميكروسكوب الإلكترونى الماسح.

وكانت النتيجة كالتالى فى الجهة التى تم غلقها زادت الخلايا الكأسية المفرزة للمخاط ونقص عدد الخلايا الهدبية بالمقارنة مع جهة الأنف المفتوحة التى زاد تيار الهواء فيها واختفت الخلايا الهدبية وتحول النسيج الأنفى العادى إلى نسيج طبقى حرشفى سميك مع زيادة الخلايا الكاسية.