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Ultrastructural ciliary findings in nasal obstructive diseases*

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SUMMARY

Specific ultrastructural findings have widely been described in case of obstructive nasal diseases due to congenital defects. Ciliary impairment has in particular been observed as the main pathological feature in these conditions.

In this study, nasal mucosal samples from different pathologies have been collected via the "brushing" technique and analysed by transmission electron microscopy. TEM analysis was focused on specific features, such as the numerical array of peripheral and central doublets of the cilium axoneme, including eventual microtubular disarrangement; partial or total loss of inner and/or outer dynein arms; defects of radial spokes and nexin links; disorientation of the ciliary axis in closely adjacent cilia, calculating the angle between the line crossing the central microtubular core and the horizontal ciliary axis and compound cilia (CC). Statistical comparison was carried out between study and control groups.

A significant incidence of organic ciliary defects was found not only in patients with inflammatory processes, but mostly in those supposed to have a long-lasting nasal respiratory disease due to mechanical stenosis in relation to septum deviation and turbinate hypertrophy. Prevalence and percentage of compound cilia were instead more related to inflammatory conditions.

The "brushing" technique can be considered an easy and reliable method for the assessment of the condition of the nasal mucosa. According to the findings derived from this study, mechanical nasal obstruction seems to cause major alterations on the nasal ciliary arrangement, thus determining a functional impairment on the whole nasal function.

Key words: nose, transmission electron microscopy (TEM), cilia, nasal obstruction, brushing

INTRODUCTION

Alterations of nasal ciliary function, such as those caused by congenital defects, e.g. the "immotile cilia syndrome", and by primary ciliary dyskinesia, have widely been investigated and correlated with specific ultrastructural findings: ciliary and hair cell loss [1-3] absence of dynein arms [2,4,5], defective radial spokes [6] and transposition of ciliary microtubules [7]. Ciliary functional incoordination, expressed by transposition of ciliary microtubules, has also been reported as further cause for the immotile cilia syndrome [8]. Ciliary dysfunction may lead to development of bronchiectasis and idiopathic bronchiectasis has been associated to a cyst-like structure within the ciliary shafts [9].

The clinical correlate for a genetically-impaired muco-ciliary function is the alteration of muco-ciliary clearance [10-13]. Muco-ciliary impairment due to chronic nasal pathologies has also been demonstrated and put in correlation with ciliary ultrastructural defects [14-16]. Since in chronic nasal pathologies the microtubular defects are not as consistently present as in primary ciliary dysfunctions, it has been hypothesized that they could be produced by infection with consequent alteration of muco-ciliary transport [17]. The typical ciliary alteration of inflammatory reactions is represented by the composed cilia, i.e. several axonemas lined by the same basal membrane [18]. This finding has been evidenced in asthma [19] and chronic rhinitis [20] with high incidence of muco-ciliary transport impairment [21,22].

Ultrastructural studies of ciliary morphostructure in specific chronic inflammatory nasal pathologies are scanty [23,24], while more attention has been paid to the recovery patterns of nasal mucosa after endoscopic nasal surgery [25-27].

This study has been designed in order to get evidence of correlations between qualitative and quantitative ultrastructural defects of ciliary unit and nasal obstructive diseases of different etiology, via an ultrastructural analysis of brushing samples

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from different conditions, i.e. normality, septum deviation, turbinate hypertrophy and osteo-meatal blockage sinusitis.

MATERIAL AND METHODS

Patients

Eighty-nine patients (55 males, 34 females) aging from 30 and 60 years (mean 45 DS ± 15) with nasal obstructive disease requiring surgery have been recruited for this study. According to the aetiology of the respiratory disease, they were further classified in three groups: A) osteo-meatal blockage sinusitis (34 patients); B) septum deviation (24 patients); C) turbinate hypertrophy (31 patients). Twenty normal subjects have also been identified on the basis of negative history and clinical exam for nasal pathology and allergy, and were included in this study as control group (D) (Table 1).

Samples

In all the subjects a cytological sample has been obtained using the "brushing" technique. This technique consists in the insertion of a brush-tip instrument at the level of the middle turbinate and in its rotation in order to collect mucosal sample. It has been carried out without local anaesthesia either before the surgical procedure or in the office for the control group.

Transmission Electron Microscopy

Each sample was immediately fixed by direct immersion of the brush-tip in a saline phosphate buffered 2% glutaraldehyde solution and stored at 4°C. Cytological samples were then post-fixed in 1% osmium tetroxide for 1 hour at 25°C, stained in 0.1% tannic acid for 30 minutes at 25°C, dehydrated in aceton, and embedded in Epon 812. Ultra-thin sections (70 to 90 µm), cut at the level of the central core of each sample, were then post-stained with lead cytrate and uranyl acetate for TEM observation (Philips Morgagni, Fei Co., Hillboro, OR, USA). For each sample at least 150 transverse sectioned cilia randomly taken from 20 different ciliated cells have been considered for evaluation.

The following ultrastructural features have been taken into account:

a) numerical array of peripheral and central doublets of the cilium axoneme, including eventual microtubular disarrangement;
b) partial or total loss of inner and/or outer dynein arms;
c) defects of radial spokes and nexin links;
d) disorientation of ciliary axis in closely adjacent cilia, calculating the angle between the line crossing the central microtubular core and the horizontal ciliary axis;
e) Compound cilia (CC).

Quantitative analysis of the incidence of the defects in each group and for CC mean percentage value in all groups has been performed.

An arbitrary grading scale for quantification of ultrastructural anomalies has been generated, where up to 5% ciliary defect was considered normal (Grade 0), while more than 5% ciliary defect was considered pathological (Grade 1). Composite cilia (CC) were graded as 0 when absent, as 1 if present.

Grade 1 (pathological) incidence in the different study groups for all parameters has been calculated. CC were also quantified as mean percentage value for each patient in all the groups.

Statistics

For statistical evaluation, Pearson's chi-square has been used for comparison of the paramethers between study and control groups. Variance analysis (Anova) was used for statistical difference of mean percentage CC value in all study groups.

Table 1. Demographical data of the study and control groups (Pearson's chi square).

Group	**n**	**M/F ratio**	**Age**
Osteo-meatal blockage-related sinusitis	34	15/19	48.1 ± 15.1
Septal deviation	24	16/8	46 ± 15.7
Turbinate hypertrophy	31	24/7	37.1 ± 14.7
Control	20	6/14	38.7 ± 21.7

RESULTS

The incidence of ultrastructural defects in the study and control groups is reported in Table 2.

Table 2. Percentage of incidence of ultrastructural defects in the study (A,B,C) and control (D) groups. a= numerical array of peripheral and central doublets of the cilium axoneme including eventual microtubular disarrangement; b= partial or total loss of inner and/or outer dynein arms; c= defects of radial spokes and nexin links; d= disorientation of ciliary axis; CC= compound cilia.

		Group A	Group B	Group C	Control Group
ULTRASTRUCTURAL DEFECTS	a	41.7	83.3	58.1	12.5
	b	29.4	75	35.5	6.2
	c	79.4	66.7	64.5	31.2
	d	41.2	37.5	41.9	25
	e	38.2	33.3	51.6	6.2

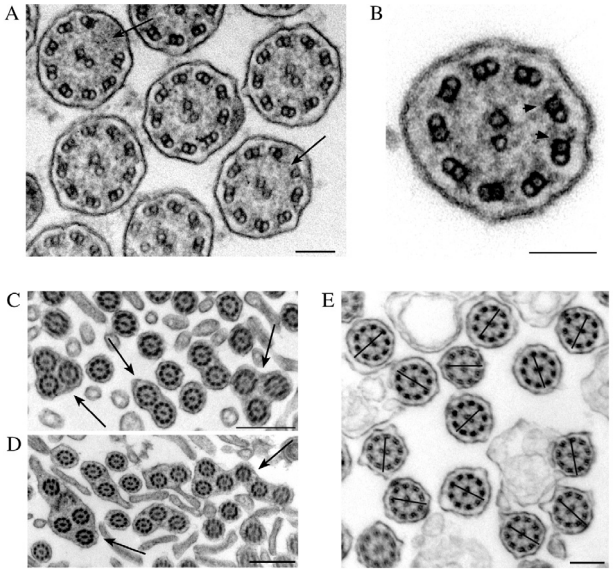

Figure 1. Transmission electron micrographs of cross-sectioned nasal cilia showing ultrastructural defects. (A) Loss of peripheral microtubules (arrows); bar = 100 nm; (B) Partial absence of inner dynein arms (arrowheads); bar = 100 nm; (C and D) Compound cilia (arrows); bars =500 nm; (E) Altered orientation of the central microtubules in closely adiacent cilia; bar =200 nm.

The analysis of microtubular arrangement (***a***) revealed as prevalent defect the presence of single tubules in the peripheral zone, most frequently in group B (83.3%), and less in group A (47.1%) and C (58.1%) (Figure 1A).

Alteration of inner and outer dynein arms (***b***) was more frequent in group B (75%) and less in group A (29.4%) and C (35.5%) (Figure 1B).

Radial spokes and nexin links (***c***) were present in all the study groups, with more prevalence in group A (79.4%) and less in group B (66.7%) and C (64.5%).

Orientation of central microtubules in closely adjacent cilia (***d***) showed in all groups a similar incidence, not statistically different from the control group (Figure 1E).

Quantitative analysis of compound cilia (Figures 1C-D) mean percentage value showed a significant difference among groups and the highest value in group C (Figure 5).

Comparison between each study group and control group for all ultrastructural features

From a statistical point of view, Group B incidence was significantly different from Group D for ***a*** ($p<0.01$), ***b*** ($p<0.01$), c ($p<0.05$) and ***CC*** ($p<0.05$), while not significantly different for ***d*** (Figure 2).

Group C was significantly different from group D for incidence of ***a*** ($p<0.01$), ***b*** ($p<0.05$), ***c*** ($p<0.05$) and ***CC*** ($p<0.05$) and not significantly different for ***d*** (Figure 2).

Group A was significantly different from group D for ***a*** ($p<0.05$) and for ***c*** ($p<0.01$) (Figure 3).

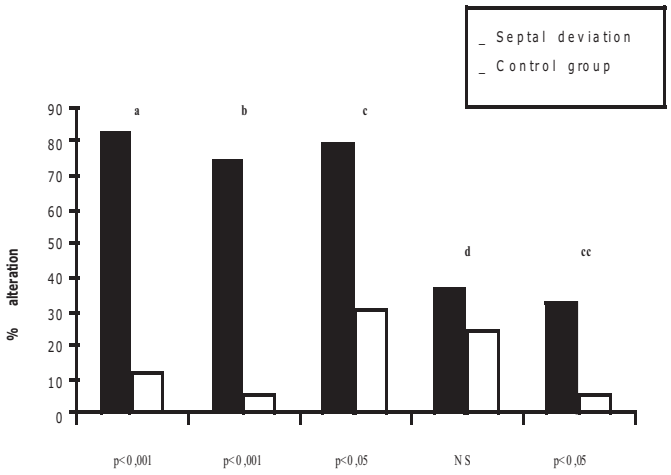

Figure 2. Comparison between septal deviation and control group for all ultrastructural parameters.

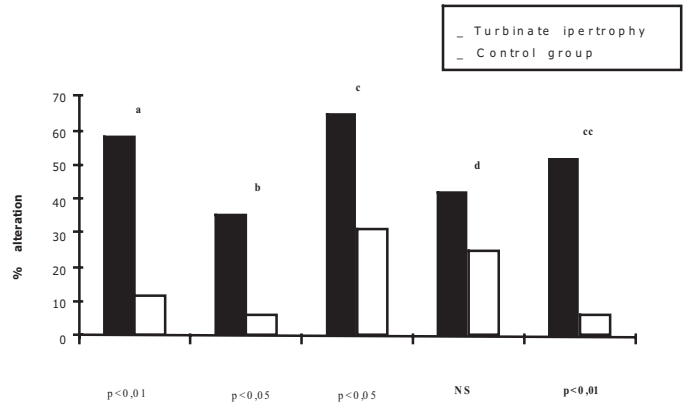

Figure 3. Comparison between turbinate hypertrophy and control group for all ultrastructural parameters.

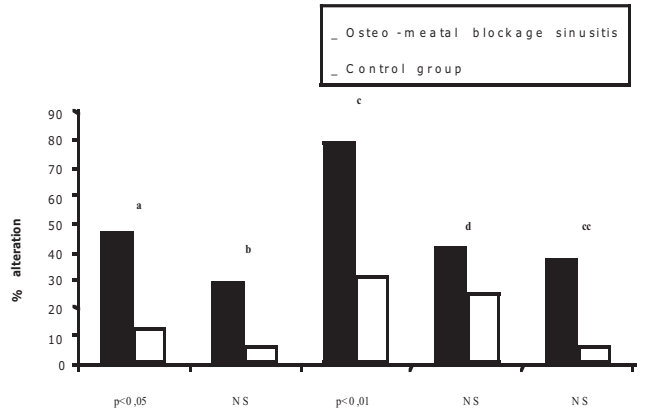

Figure 4. Comparison between osteo-meatal blockage sinusitis and control group for all ultrastructural parameters.

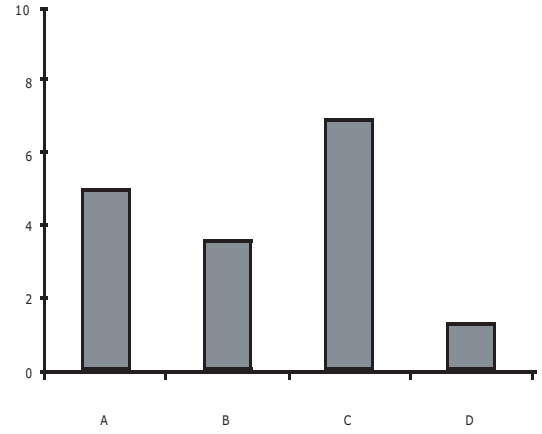

Figure 5. Quantitative analysis of cilia mean percent value in each group (Anova: p=0,001). a: microtubules arrangement; b: dynein arms; c: radial spokes; d: microtubules orientation.

Comparison among groups for percentage of defect frequency

CC feature incidence among groups showed the defect to be more frequent in group C than in A, B and control groups.

DISCUSSION

Alterations of ciliary ultrastructure have been correlated to impairment of nasal mucociliary clearance (NMCC) impairment [14-17] and described as primary ciliary defects, e.g. in the "immotile cilia syndrome", or as acquired ultrastructural modifications occurring in nasal inflammatory chronic diseases [28,29]. In fact, it has recently been suggested that although most patients with ciliary ultrastructural defects may have primary defects [30], ciliary alterations might also be present in healthy subjects [31,32], in children exposed to chronic and sequential air pollutants [23], or related to aging [24,33]. In addition, quantitative and qualitative changes in nasal respiratory function may be induced also by anatomical modifications, damage from recurrent infections, trauma and exposure to external toxin and other unknown factors. Therefore, it is quite difficult to demonstrate if a single nasal pathology directly or indirectly leads to ciliary dysfunction when all these factors merge.

Aim of the present study was to assess the occurrence and characteristics of ciliary ultrastructural defects in nasal respiratory diseases. In particular, object of the investigation were two types of common nasal obstructive diseases, such as septum deviation and turbinate hypertrophy, which were compared to the osteo-meatal blockage-related sinusitis, as well as to a group of healthy, non smokers patients recruited on the base of negative anamnesis and clinical signs for upper and lower respiratory disease, aspirine allergy, aging between 30 and 60 years. A significant difference between septum deviation, turbinate hypertrophy and the control group was observed for all ultrastructural defects examined, except for the microtubular orientation, whereas osteo-meatal blockage-related sinusitis and control group findings showed significant differences only for the tubular configuration and connective spokes.

As far as the prevalence of ultrastructural defects in the 9+2 configuration is concerned, dynein arms, radial spokes and nexin links variations were more represented in septum deviation and turbinate hypertrophy compared with osteo-meatal blockage sinusitis. Compound cilia were instead more frequent in turbinate hypertrophy and osteo-meatal blockage-related sinusitis, and less in septum deviation. These findings could be explained also on the basis of the very few previous studies focused to the pathophysiological and clinical aspects correlated with ultrastructural ciliary defects [3,6,9,17,19,23,29]. In those studies, in fact, transmission electron microscopy allowed to demonstrate a remarkable number of defects which, in accordance with the clinical manifestation, could be distinguished in: i) a chronic inflammatory pattern, represented by swollen and/or compound cylia, missing or supernumerary peripheral microtubules or pairs; and ii) a more severe pattern probably related to primary ciliary dyskinesia, characterized by missing or supernumerary central microtubules or pairs, central translocation of peripheral pairs and lost or shortened dynein arms [2,34-36]. It is noteworthy to remind that both in primary and secondary ciliary dyskinesia a normal ultrastructure with abnormal orientation of the central microtubules has also been described [8-10], thus suggesting that dysinchronia may be the only pathophysiological expression of ciliary dysfunction in different clinical manifestations.

Literature which correlates ultrastructural defects in nasal pathologies is scanty and mostly addressed to chronic inflammatory diseases [3,6,9,17,19,23,29]. In contrast with previous reports, the results obtained in the present study showed a significant incidence of organic ciliary defects not only in patients with inflammatory processes (Group A), but mostly in those supposed to have a long-lasting nasal respiratory disease due to mechanical stenosis (Group B and C). Both the prevalence and percentage of compound cilia appeared to be more related to inflammatory conditions (Group A), although not pathognomonic, since they were also significantly present in patients without evidence of active inflammatory processes. Since compound cilia represent the product of elastasis enzymatic action during chronic respiratory mucosa inflammation [10,21], their incidence in all study groups and the mean percentage value for patient would suggest a role of recurrent or previous inflammatory diseases in the majority of the patients, as a consequence of nasal stenosis.

The recovery of respiratory mucosa after endoscopic sinus surgery (ESS) has recently been documented, stressing the role of altered mucociliary transport to produce ultrastructural modifications of nasal ciliary mucosa during chronic sinusitis [26]. In our series of osteo-meatal blockage-related sinusitis, compound cilia were less represented than in turbinate hypertrophy, suggesting that both the inflammatory process and the alteration of mucociliary transport occur inside the paranasal cavities and

affect the nasal mucosa to a minor degree.

In contrast to previous reports, microtubular disarrangement, which is considered the dysfunctional cause for desynchronized ciliary beat, was not significantly different between the pathological and the control groups, even in presence of dynein arms defects which are presumed to induce a swinging of microtubule and distortion of axonemic structure [37]. Moreover, in no case it was possible to observe the association of this functional defect to a normal ultrastructure.

The organic ciliary defects are usually considered to be correlated to the duration of the respiratory dysfunction, which basically prevails in septum deviation, respect to the inflammatory process involved in turbinate hypertrophy and in osteo-meatal blockage-related sinusitis. In these latter, compound cilia formation represents the more common finding.

It is possible to conclude that all the ultrastructural defects relative to ciliary configuration in patients with nasal respiratory stenosis are likely to be secondary in origin, considering the functional recovery observed after surgery. Organic defects and compound cilia have been reported in all study groups with significant difference of incidence in respect to healthy subjects: the first one with probable correlation with the duration of the respiratory disease, the second one due to chronic inflammatory disease. Longitudinal TEM analysis of brushing samples of nasal mucosa from patients with worse ultrastructural defects could be helpful to better elucidate this issue. Further studies are needed in order to elucidate the correlation between mechanical nasal stenosis, possible muco-ciliary transport alteration and ciliary ultrastructural defects, especially in patients with poor functional post-operative recovery.

REFERENCES

1. Tsang KWT, Zheng L, Tipoe G (2000) Ciliary assessment in bronchiectasis. Respirology 5: 91-98.
2. Pifferi M, Cangiotti AM, Ragazzo V, Baldini G, Cinti S, Boner AL (2001) Primary ciliary dyskinesia: diagnosis in children with inconclusive ultrastructural evaluation. Pediatr Allergy Immunol 12: 274-282.
3. Rossman CM, Forrest JB, Newhouse MT (1984) Nasal ciliary ultrastructure and function in patients with primary ciliary dyskinesia compared with that in normal subjects and in subjects with various respiratory diseases. Am Rev Respir Dis 129: 161-167.
4. Afzelius BA (1976) A human syndrome caused by immotile cilia. Science 193: 317-319.
5. Pedersen M, Mygind N (1976) Absence of axonemal arms in nasal mucosal cilia in Kartagener's syndrome. Nature 262: 494-495.
6. Sturgess JM, Chao J, Wong J, Aspin N, Turner JAP (1979) Cilia with defective radial spokes, a cause of respiratory disease. N Engl J Med 300: 53-56.
7. Sturgess JM, Chao J, Turner JAP (1980) Transposition of ciliary microtubules. A further cause for 'immotile cilia syndrome'. N Engl J Med 303: 318-322.
8. Rautiainen M, Collan Y, Nuutinen J, Afzelius A (1990) Ciliary orientation in the 'immotile cilia' syndrome Eur Arch Othorinolaryngol 247: 100-103.
9. Tsang K, Tipoe G, Sun J et al. (2000) Severe bronchiectasis in patients with 'cystlike' structures within the ciliary shafts. Am J Respir Crit Care Med 161: 1300-1305.
10. Turner JAP, Christopher WB, Corkey B, Jonh YC Lee, Levision H, Sturgess M (1981) Clinical expression of immotile cilia syndrome. Pediatrics 67: 805-810.
11. Greenstone M, Rutman A, Dewar A, Mackay I, Cole PJ (1988) Primary ciliary dyskinesia: cytological and clinical features. Quarterly J Med, New Series 67: 405-423.
12. Rautinien M, Nuutinen J, Collan Y (1991) Short nasal respiratory cilia and impaired cilia and impaired mucociliary function. Eur Arch Otorhinolaryngol 248: 271-274.
13. Houtmeyers SE, Gosselink R, Gayan-Ramirez G, Decramer M (1999) Regulation of mucociliary clearance in health and disease. Eur Respir J 13: 1177-1188.
14. Toremalm NG (1984) Mucociliary function in chronic infection. Rhinology 22: 111-113.
15. Konietzko N (1986) Mucus transport and inflammation. Eur J Respir Dis 69 (Suppl 147): 72-79.
16. Majima Y, Sakakura Y, Matsubara T, Miyoshi Y (1986) Possible mechanism of reduction of nasal mucociliary clearance in chronic infection. Clin Otolaryngol 11: 55-60.
17. Carson JL, Collier AM, Hu SS (1985) Acquired ciliary defects in nasal ephitelium of children with acute viral upper respiratory infection. N Engl J Med 312: 463-468.
18. Ailsby RL, Chadially FN (1973) Atypical cilia in human bronchial mucosa. J Pathol 109: 75-78.
19. Cutz E, Levison H, Cooper DM (1978) Ultrastructure of airways in children with asthma. Histophatol 2: 407-421.
20. Takasaka T, Sato M, Onodera A (1980) Atypical cilia of the human nasal mucosa. Ann Otol Rhinol Laryngol 89: 37-45.
21. Toskala E, Nuutinen J, Rautiainen M, Torkkeli T (1995) The correlation of mucociliary transport and scanning electron microscopy of nasal mucosa. Acta Otolaryngol (Stockh) 115: 61-65.
22. Yang-Gi M, Jin Sung S, Seung-Ho C, Je G Chi, Chul-Jong Y (1995) Primary ciliary dyskinesia: ultrastructural defects and clinical feature. Rhinology 33: 189-193.
23. Calderon-Garciduenas L, Valencia-Salazar G, Rodriguez-Alcaraz A, et al. Ultrastructural nasal pathology in children chronically and sequentially exposed to air pollutants. Am J Resp Cell Mol Biol 24: 132-138.
24. Ho JC, Chan KN, Hu WH, et al. (2001) The effect of aging on nasal mucociliary clearance, beat frequency and ultrastructure of respiratory cilia. Am J Resp Crit Care Med 163: 983-988.
25. Stammberger H, Hawke M (1993) Essentials of functional endoscopical sinus surgery. St Louis, Mo, Mosby-Years Book Inc..
26. Fang SY (1994) Normalization of maxillary sinus mucosa after FESS: a prospective study of chronic sinusitis with nasal polyps. Rhinology 32:137-140.
27. Yongqing G, Yuichi M, Masahiko H, Shinji S, Yasuo S (1997) Effect of Functional Endoscopic Sinus Surgery on maxillary sinus mucosa. Otolaryngol Head Neck Surg 123: 1097-1100.
28. Lewis FH, Beals TF, Carey TE, Baker SR, Mathews KP (1983) Ultrastructural and functional studies of cilia from patients with asthma, aspirin intolerance and nasal polyps. Chest 83: 487-490.
29. Giannessi F, Fattori B, Ursino F, Giambelluca MA, Soldani P, Scavu-Ruffoli R (2003) Ultrastructural and ultracytochemical study of the human nasal respiratory epithelium in vasomotor rhinitis. Acta Otolaryngol 123: 943-949.
30. Tsang KWT, Sun J, Lam WK (1998) Ultrastructural and functional ciliary assessment in Chinese patients with bronchiectasis. Respirology 3 (Suppl): A4.
31. Eiongh R, Rutland J (1955) Ciliary defects in healthy subjects, bronchiectasis and primary ciliary dyskinesia. Am J Respir Crit Care Med 151: 1559-1567.
32. Chilvers MA, Rutman A, O' Callaghan C (2003) Functional analysis of cilia and ciliated epitelium ultrastructure in healthy children and young adults. Thorax 58: 333-338.
33. Schrodter S, Biermann E, Halata Z (2003) Histological evaluation of age-related changes in human respiratory mucosa of the middle turbinate. Anat Embryol 207: 19-27.
34. Meeks M, Bush A (2000) Primary ciliary dyschinesia (PCD) Pediatr Pulmonol 29: 307-316.
35. Rossman CM, Lee RMKW, Forrest JB, Newhouse MT (1984) Nasal ciliary ultrastructure and function in patient with primary ciliary dyskinesia compared with that in normal subjects with various respiratory diseases. Am Rev Respir Dis 129: 161-167.

36. Mierau GW, Agostini R, Beals TF (1992) The role of electron microscopy in evaluating ciliary dysfunction. Report of workshop. Ultrastruct Pathol 16: 245-254.
37. Lurie M, Rennert G, Goldenberg S, Rivlin J, Greenberg E, Katz I (1992) Ciliary ultrastructure in primary ciliary dyskinesia and other chronic respiratory conditions: The relevance of microtubular abnormalities. Ultrastruct Pathol 16: 547-553.

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