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Nasal ciliary studies in children with chronic respiratory tract symptoms*

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SUMMARY

Ciliary abnormalities include a range of morphological dysfunctions resulting in dysmotility. These typically manifest with upper and lower respiratory symptoms later in the infancy and early childhood. The diagnosis is based on ciliary studies i.e. measurement of ciliary beat frequency (CBF). We present a series of 67 children who had nasal biopsy and ciliary studies done between 1993-2002.

There were 44 boys and 23 girls of age between 1 to 17 years. In 49 (73%) cases indication of ciliary studies was chest symptoms. There were six diagnostic categories: asthma, recurrent chest infections, bronchiectasis, rhinosinusoitis, dextrocardia and prematurity.

Fourteen patients (20 %) had dextrocardia and of these 9 had no measurable beating cilia. In the rest 5 CBF ranged 8-12.7Hz. If the patients with dextrocardia are excluded, 5 of the 53 (10 %) did not have any ciliary activity and in remaining CBF ranged 5.3-19.7Hz.

Our results showed a significant number of children with immotile cilia had associated dextrocardia. In the absence of situs inversus index of suspicion should be very high to detect these cases early. Otolaryngologists can play a key role in diagnosis because of an easy access for nasal biopsy, which is much simple than bronchial biopsies.

Key words: nasal epithelial cells, cilary beat frequency, immotile cilia, dextrocardia

INTRODUCTION

The respiratory tract and middle ear cleft are lined by a secretory epithelium, mostly pseudostratified columnar type. The ciliated cells are characterized by long cytoplasmic projections and numerous microvilli. They have between 20 and 200 cilia per cell (Serafini et al., 1977). The ciliary beat cycle consists of an effective stroke and a recovery stroke. During the effective stroke the cilium is fully extended, penetrates the mucous and sweeps the mucous layer. In recovery stroke it is bent and flexed. The cilia beat in a coordinated metachronous fashion, coupling with overlying mucus. Ciliary beat frequency (CBF) is normally 7-14 beats per second, and the rate is temperature dependent. This is independent of nervous control, but is influenced by radiation, pH, viscoelastic properties of mucus, drugs as well as by disease. Measurements of CBF can help in diagnosis of various conditions. A number of techniques have been used over the years and we used a photomultiplier previously (Robson et al., 1993) We take measurements now with a photodiode, and have developed a program written in G, which works under Windows.

Ciliary abnormalities include a range of morphological dysfunctions resulting dysmotility. These typically manifest with upper and lower respiratory symptoms later in infancy and early childhood. These are a well-recognized cause of secretion retention and pneumonia in the immediate neonatal period (Ramet et al., 1986). Otitis media with effusion is the common otological manifestation (el-Sayed et al., 1997). The management of children with refractory symptoms may require the exclusion of ciliary abnormality as part of the diagnostic evaluation.

Cytology from the nose is easy for an otorhinolaryngologist. A biopsy is taken at the same time and morphological assessment may be made using electron microscopy (Robson et al., 1993). Our department has developed a ciliary service for cases referred by the chest physicians, neonatologists and ENT surgeons at Birmingham Children's Hospital. We present the results on a series of children between 1993-2002.

METHODS AND PATIENTS

Sixty-seven children with possible ciliary abnormalities were admitted under the care of the second author. Sixty children had their brushings and biopsies taken under general anaesthesia. Six children who were in neonatal critical care units had a brushing taken alone. Eighteen children had fibre-optic bron-

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choscopy at the same time, performed by the chest physician.

Procedure

Neither topical anesthesia, nor vasoconstrictive agents were used for the procedures. We have described out technique previously (Robson, et al., 1992). A 1.73 mm cytology brush (Wilson-Cook) is inserted in the nasal cavity. It is moved anteroposteriorly over the inferior turbinate. The sample of epithelium is then dislodged into 2 mls of culture medium in a sterile container (Medium 199 with HEPES and Earle's salts, Sigma). The biopsy is taken from the other nasal cavity. A 2mm cupped aural granulations forceps is used to take it from the middle of the inferior turbinate. It is transferred immediately to the laboratory for fixation.

The fragments of nasal epithelium are transferred from the culture medium with a pipette and deposited on the cover slip. The slide is placed under an electronically controlled warm stage mounted on a Zeiss Universal Microscope. The temperature of the measurement is recorded.

CBF is measured photometrically. Beating cilia are clearly seen at a magnification of X 400 by bright light illumination. These are targeted by a scope that allows positioning of the beating cilia so as to obstruct the passage of light through a small diaphragm (3.14 m^2). The field is selected to allow a single cilium to beat across its aperture. A photodiode attached to the head of the microscope transduce changes in light intensity into electrical signal. The transduced electrical signal is amplified and displayed on the monitor. The signal is analyzed by a computer programme using a Fast Fourier Transform. The standard deviation and standard error of the mean are calculated of at least ten measurements. All samples are evaluated by the second author.

RESULTS

Sixty-seven children underwent ciliary studies. In 49 (73 %) patients indication for ciliary studies were chest symptoms. Table 1 shows a range of medical disorders in these patients. There were six diagnostic categories. Two patients did not fit into these, one had inverted abdominal viscera and the other had failure of development of the lungs. All patients who had no obviously beating cilia but had levocardia were retested.

Table 1. Diagnostic categories.

Diagnosis	No (%)	CBF=0
Asthma	14 (21)	1
Recurrent chest infections	18 (27)	2
Bronchiectasis	7 (10)	0
Failure to thrive and prematurity	6 (9)	2
Dextrocardia	14 (21)	9
Rhinosinusitis	6 (9)	0
Others	2 (3)	0
Total	67 (100)	14

There were 44 boys and 23 girls. The age ranged from <1 to 17 years with median of 7 years. Six of the babies were investigated on the neonatal unit with either prematurity or failure to thrive and were grouped together.

Fourteen patients (20 %) had dextrocardia and of these, 9 had no obvious measurable beating cilia. Two of these patients had some activity but it was neither coordinated nor measurable. The range of CBF in patients with dextrocardia was from 8 to 12.7Hz.

If the patients with dextrocardia are excluded, 5 of the 53 remaining (10 %) patients did not have any evidence on repeated testing of any ciliary activity. The ciliary beat frequency ranged from 5.3 - 19.7Hz (all patients with beating cilia), and the mean and median were 10.4Hz and 10Hz respectively. As the beat was measured at room temperature, this variation is acceptable (Figure 1).

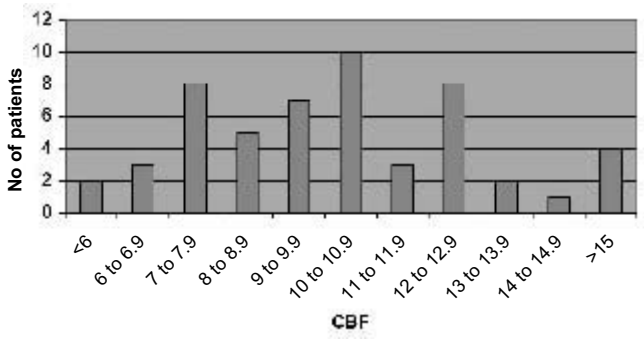

Figure 1. The distribution of ciliary beat frequency in 53 patients.

DISCUSSION

Primary ciliary dyskinesia (PCD) is a heterogeneous disease with impaired mucociliary transport leading to upper and lower respiratory disorders, hearing impairment and male infertility. Afzelius (1976) noted two infertile brothers had normal sperms in all respect except absent dynein arms, and both had chronic bronchitis and chronic sinusitis. PCD can only be diagnosed by clinical features together with functional and structural analysis of the cilia. It is now recognized that there is a spectrum of severity of changes in ciliary dysmorphology and ciliary function.

Cilia have been identified in the respiratory tract as early as seventh week of gestation (Moscoso et al., 1988). A popular view is that the alignment of the heart and gastrointestinal tract may be determined by intact ciliary function. The heart could go to the left or right by chance and this accounts for immotile cilia in patients with levocardia. Patients with dextrocardia have variable degrees of inversus and some patients with dextrocardia have normal beating cilia so the hypothesis is not tenable. We found that five of the patients with dextrocardia had normal beating cilia and this corresponds to the published texts of 50 percent (Roberton et al., 1999).

PCD is unlikely to be suspected in infants in absence of dextrocardia and situs inversus, unless there is an affected sibling.

Clinical suspicion is important, as are close links with the paediatric disciplines. The diagnosis is confirmed by assessing ciliary function and ultrastructure. In adults, nasal biopsy can be done in outpatients (Friedman, 2000) children nasal biopsy is taken under anesthetic. It is important not to use any topical anesthetic or vasoconstrictive agent, as it will affect the viability of ciliated cells.

We have found five patients with levocardia who have ciliary dysmotility, which is just less than 10 percent of the population studied. Our clinical service developed out of a research project and patients are referred throughout the West Midlands. Teamwork allows the chest physician to preform a fiberoptic bronchoscopy at the same time and undertake bronchiolar lavage. While rare, ciliary disease must be identified early, as aggressive physiotherapy will prevent permanent chest damage. The middle ear disease is often refractory to treatment and ventilation tubes discharge persistently.

CONCLUSIONS

Ciliary motility abnormalities can be diagnosed in children with the help of light microscopy techniques and the measuring ciliary beat frequency. Our results showed a significant number of children with immotile cilia had associated dextrocardia. In absence of situs inversus the index of suspicion should be very high to detect these cases early.

Otolaryngologists can play a key role in diagnosis because of an easy access for nasal biopsy, which is much simple than bronchial biopsies.

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