CASE REPORT

Function and structure of cilia in the Fallopian tube of an infertile woman with Kartagener’s syndrome


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In Kartagener’s syndrome (KS), primary defects of the ciliary axoneme cause dyskinetic ciliary motion. Because ciliary motion is an important factor in normal ovum transport, ciliary dyskinesia may cause infertility. On the other hand, the existence of some ciliary activity, albeit abnormal, may account for fertility in some women with KS. In this case study, an infertile woman diagnosed with KS had normal results in all usual infertility tests. Biopsies of tubal mucosa were obtained at laparoscopy for ovum recovery during an in-vitro fertilization cycle. Ciliary activity, measured by laser light-scattering spectroscopy, was detected in all tubal specimens; however the majority of regions sampled showed no activity. In active regions, beat frequency ranged from 5 to 10 Hz, ~30% of normal. Electron microscopy showed similar morphological defects in both tubal and nasal mucosa. The number of cilia per cell was ~20% of normal. The major ultrastructural abnormality of cilia was an absence of the central microtubules. The only demonstrable explanation for this patient’s infertility was primary ciliary dyskinesia associated with KS.

Key words: ciliary dyskinesia/Fallopian tube/Kartagener’s syndrome/laser spectroscopy

Introduction

Numerous experiments in a variety of animal models, each illustrating the primary role of cilia in transporting ova through the distal portion of the oviduct, have emphasized the importance of ciliary motility in normal mammalian reproduction (Halbert, 1983; Halbert et al., 1989). At ovulation the egg is surrounded by mucus, and the primary means by which the egg is moved through the distal tube is mucociliary transport, not unlike that in the respiratory tract (Halbert, 1983). Impairment of ciliary activity, commonly associated with infection and rarely with congenital defects, has been proposed as a cause of female infertility (Halbert, 1983; Bateman et al., 1987).

Kartagener’s syndrome (KS), which is characterized by the clinical presentation of chronic sinusitis, bronchiectasis and situs inversus, is believed to be caused by a primary defect of the cilia, and KS is thus regarded as a subgroup of primary ciliary dyskinesia (PCD). The combined presence of severe impairment of airway mucociliary clearance and ultrastructural defects in the ciliary axoneme initially suggested that the cilia in patients with KS were immotile (Eliasson et al., 1977). The observation that some women with KS are fertile suggested that ciliary motility is unnecessary for reproduction (Afzelius et al., 1978; Bleau et al., 1978). This hypothesis was supported indirectly by an ultrastructural study that showed an absence of dynein arms in cilia from the fimbria of a fertile KS patient (Jean et al., 1979).

Subsequent studies described variable axonemal defects as well as variable degrees of ciliary motion in KS patients (Pedersen and Mygind, 1980; Roseman et al., 1980), reopening the possibility that fertility in women with KS may after all be explained by the existence of ciliary motility, even if it is dyskinetic. Only one prior study of KS has included measurements of motility of Fallopian tube cilia, and in this case the patient’s infertility was ascribed to absolute ciliary immotility (McComb et al., 1986). In the present study of an infertile KS patient, we found ciliary motility to be substantially reduced but not totally absent. This new evidence supports the hypothesis that ciliary dyskinesia and not necessarily complete immotility may cause infertility, and it raises a question as to what degree of ciliary function is necessary to promote fertility.

Case report

The patient in the present case was a 23 year old female, gravida 0, who was unable to conceive for 3 years. She was suspected of having KS because she had a long history of chronic respiratory problems, and both her brother and mother had been diagnosed as having Kartagener’s triad (chronic sinusitis, bronchiectasis, and situs inversus).

The results of the couple’s basic infertility tests were normal, including biphasic basal body temperature charts with a normal ovulatory progesterone concentration, normal semen analysis and post-coital test, and normal appearance of uterine/pelvic anatomy at hysterosalpingography and laparoscopy. Her infertility was ascribed to tubal dysfunction due to KS, and she was subsequently admitted to the University of Washington’s in-vitro fertilization (IVF) programme.

Ovum recovery was accomplished by mini-laparotomy after ovarian stimulation with clomiphene citrate/human menopausal gonadotrophin (HMG). Then bilateral, full-thickness 2×8 mm tubal biopsies were excised by sharp dissection from left fimbria and right ampulla. Motility of the cilia in these specimens was measured by laser light-scattering spectroscopy. Morphology of the tubal mucosa was assessed by scanning and transmission electron microscopy. Biopsies of nasal...
mucosa were also obtained for morphological comparison with the tubal mucosa.

For the motility studies, the tubal specimens were washed gently with physiological saline solution to remove debris from the ciliated surface. Specimens were then dissected into 2 x 3 mm pieces and mounted in separable tissue culture chambers similar to those originally described by Rose (1954), which were filled with modified Eagle's medium. Two Rose chambers were prepared using tissue from fimbria and three from ampulla. Following a preliminary inspection of ciliary motility with the laser system, the Rose chambers were stored in an incubator at 37°C until the experiments were conducted the following day.

The laser light-scattering system used to measure ciliary beat frequency was described previously (Holloway et al., 1988). Briefly, monochromatic light from a 5 mW He/Ne laser was directed through an optical fibre to illuminate the mucosal surface of the tissue. Light back-scattered from the tissue was conducted through a parallel optical fibre to a photodiode, and spectral analysis of the resultant electronic signal was performed using computer software (ASYST, MacMillan Software). Ciliary beat frequency was measured as the peak of the frequency power spectrum resulting from a fast Fourier transform of the photodiode signal.

At the time of study with the laser system, the Rose chambers were placed in an air-filled, temperature-regulated (37°C) environmental chamber. After allowing 15–20 min for equilibration, ciliary beat frequency was determined for each of three to five sites selected at random from each tissue segment. In order to obtain visual confirmation of ciliary beating, the Rose chambers were also observed under an inverted phase-contrast light microscope equipped with a temperature-regulated (37°C) stage.

Tissue specimens from this woman’s salpingeal and nasal mucosa were prepared for electron microscopy according to methods described previously (Patton et al., 1989). Scanning electron microscopy (SEM) was used to examine the topography and ciliary density of the mucosal surface. Numbers of cilia atop single cells were counted using SEM at a magnification of ×3500. In addition, cilia were counted using several transmission electron microscopy (TEM) grids at ×3500. At least five cells were studied in each case to determine the average cilia count. For comparison, normal ciliary density was measured from electron micrographs of normal tubal specimens from nine individuals that were obtained as controls for previous studies (Patton et al., 1989). TEM was also used to assess the ultrastructure of individual cilia in cross-section.

Results

Only a small proportion of sites monitored with the laser system displayed measurable ciliary activity (e.g., five of 35 sites in one Rose chamber). Spatial discontinuity of activity was noted on specimens from both ampulla and fimbria. Ciliary beat frequency in active sites ranged from 4.7 to 9.8 Hz on fimbrial specimens, and from 5.5 to 10.5 Hz on ampullary specimens. Observations of Rose chambers under the inverted light microscope confirmed that ciliary beating was present in some regions of all specimens, as well as that the majority of regions showed no activity.

Scanning electron micrographs of the mucosal surfaces of both tubal and nasal biopsies showed irregular surface topography, sparse ciliation on individual ciliated cells, and collections of mucus and cellular debris (Figure 1). The relative number of ciliated cells appeared normal. The number of cilia on ciliated cells ranged from eight to 36 per cell in tubal specimens (average 17). Normal salpingeal mucosa had 250–300 cilia per cell. In nasal specimens, ciliary density ranged from 10 to 90 per cell (average 39). Individual cilia were clearly shorter than normal, although a quantitative comparison was not made.

Transmission electron micrographs of cross-sections through individual cilia demonstrated that dynein arms were present, although no single micrograph provided sufficient clarity to confirm that all dynein arms were present. Dynein arms appeared to lack the ‘hook’ appendage present in normal cells. The most notable observation was a uniform absence of the central pair of microtubules that is present in normal cilia (Figure 2). No apparent differences between fimbria and ampulla were seen, and nasal biopsies were similar in appearance to those from the Fallopian tube.

The patient failed to conceive on this single IVF attempt.

Discussion

This report is only the second to evaluate both the structure and function of cilia from the Fallopian tube of a patient with KS. McComb et al. (1986) attributed infertility in their case study to the absolute lack of ciliary motility associated with partial or total absence of dynein arms. Our study provides the first direct evidence of motile cilia in the Fallopian tube of a patient with KS. The motility was dyskinetic, however, with the ciliary beat frequency in active regions reduced to approximately one-third of that found in normal tubal mucosa (23.4 ± 1.5 Hz) (Patton et al., 1989), and the major ultrastructural defect associated with this subnormal motility was an absence of the central microtubules in individual cilia.

Ultrastructural defects in PCD, including KS, are heterogeneous, and associated functional defects are variable, indicating that the relationship between structural defects and dysfunction is not absolute. For example, a wide range of mucociliary clearance rates was found among different patients whose airway cilia displayed an absence of dynein arms (Barlocco et al., 1991), which is the most prevalent ultrastructural defect found in PCD. Respiratory cilia and sperm tails were both ultrastructurally normal in a male with KS who suffered from chronic bronchopulmonary disease but who was fertile and had normal sperm motility (Conraads et al., 1992). Uniform defects of the central microtubules are rare, yet our findings are consistent with those of Torikata et al. (1991), who reported two patients with a high percentage of defective central microtubules in motile respiratory cilia.

Another unusual finding in this KS patient was the abnormally low density of cilia on individual cells in both tubal and nasal mucosa. The relative abundance of cells that possessed cilia was, however, apparently normal. Chronic respiratory problems might provide an explanation for the observed airway deciliation, but there was no evidence of pelvic inflammatory disease (PID) to account for tubal deciliation. The facts that the tubal specimens were taken at mid-cycle and the ovarian function of this patient appeared normal argue against the possibility that the tubal deciliation was caused by reduced oestrogen concentrations. The cause of low ciliary density in
Ciliary dyskinesia and infertility

Figure 1. Scanning electron micrograph of mucosa from the Fallopian tube of an infertile patient with Kartagener’s syndrome. The ciliated cells (c) contained <100 cilia per cell, and individual cilia were shorter than normal. Many of the ciliated cells have been extruded from the mucosal surface, although the relative abundance of ciliated and secretory cells appeared normal. The secretory cells (s) contained thickened microvillous projections. Original magnification ×3300. Scale in lower right hand corner is 1 mm.

Figure 2. Transmission electron micrograph of cross-section through a cilium from tubal mucosa of an infertile patient with Kartagener’s syndrome. The absence of the two central microtubules is shown, giving a 9 + 0 microtubule pattern (arrows) instead of the normal 9 + 2 configuration. Dynein arms are present; however, they appear to lack the ‘hook’ appendage present in normal cells. Original magnification ×104 000. Scale in lower right hand corner is 0.25 mm.

this patient is not known, and the possibility that it is a primary condition rather than an acquired one must be considered.

Reduced ciliary motility and density could have prevented normal clearance of cell secretions and cellular debris, allowing these by-products of normal biological function to accumulate on the surface of the tubal mucosa. It is also possible that the cellular debris could have resulted from excessive cellular extrusion, which could have been caused by a variety of conditions either in vivo, such as undiagnosed tubal disease, or ex vivo, such as inadvertent mishandling of tissue after excision. Although the cause of the accumulation of cellular debris cannot be determined, inadequate ciliary clearance seems to be the most likely candidate, implicating KS as a secondary rather than primary cause of this condition. Because the mucosal surface was irrigated prior to examination with the laser, it may be presumed that the debris seen in the scanning electron micrographs had been removed and therefore did not affect the measurements of ciliary beat frequency. However, it is possible that the presence of such debris in vivo may have reduced ciliary activity even further.

Some elements of this investigation show interesting similarities to results from previous studies of female infertility associated with PID (Patton et al., 1989). In tubal biopsies from women with distal tubal obstruction, ciliary beat frequency was one-third that of normal values. The morphology of the tubal mucosa was variable in the PID patients, but no evidence of ultrastructural abnormalities in individual cilia was seen to explain the reduced motility, and even normal-appearing tubal mucosa displayed reduced ciliary activity. Historically, >70% of such patients remain infertile after successful surgical
reversal of tubal occlusion, indicating that tubal dysfunction persists, and it has been postulated that this is due to the persistence of reduced ciliary motility (Halbert, 1983).

The common feature of subnormal ciliary beat frequency in this infertile KS patient and in infertile PID patients suggests that there may be a threshold of ciliary beat frequency below which mucociliary ovum transport fails, irrespective of whether the functional defect is primary or acquired. An adequate experimental test of this hypothesis would require a large prospective study of fertile and infertile women that included, among many other important variables, measurements of ciliary beat frequency and ciliary morphology. Perhaps the strongest evidence would come from the direct observation of ciliary activity in the Fallopian tube of a fertile KS woman.

The only identifiable explanation for this patient’s ongoing infertility is ciliary dyskinesia. Although IVF, a procedure that circumvents tubal dysfunction, was unsuccessful, the success rate of this procedure on initial attempts is such (10–20% pregnancy rate per IVF attempt) that this negative result does not contra-indicate the diagnosis of primary infertility due to tubal dysfunction associated with KS.

These results of this case study support the hypothesis that ciliary dyskinesia and not necessarily complete immotility can cause infertility. The motility of cilia in the Fallopian tubes of a fertile woman with KS remains to be determined.

Acknowledgements

This study was supported by National Institutes of Health Grants RO1 HD 10988 and RO1 HD 23528. The laser light-scattering spectroscopy studies were performed in Dr Halbert’s laboratory by Richard Anderson.

References


