Functional study of nasal mucosa in endoscopic sinus surgery and its correlation to electron microscopy of cilia

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Abstract The effect of functional endoscopic sinus surgery (FESS) was evaluated in 20 patients with chronic sinusitis. The physiological function of sinus mucosa and its mucociliary transport mechanism was assessed pre and post operatively in patients undergoing FESS by performing Saccharin clearance test. The effect of functional sinus surgery on nasal mucosa was evaluated using scanning electron microscopy and study of the ultrastructure of cilia in pre and post operative patients and correlation with histopathological examination. The mucociliary transport mechanism of the sinus mucosa improved 6 weeks following surgery. Histopathologically there was evidence of improvement in the ciliary population and decrease in inflammation, ulceration, fibrosis, vascular congestion, edema, squamous cell metaplasia, basement membrane thickening and polyp formation in comparison to the diseased mucosa sampled preoperatively. Scanning electron microscopic examination revealed considerable increase in the ciliary area as well as the orientation of cilia. Subjective improvement 6 weeks following surgery was confirmed by decrease in saccharin test time. This study revealed that FESS is an effective surgical procedure in chronic sinusitis and improves the overall function of the sinuses

Keywords Functional Endoscopic Sinus Surgery · FESS · Mucociliary transport · Saccharin test · Ultra structure of cilia · Nasal mucosa · Chronic Sinusitis

Introduction

Chronic sinusitis is defined as persistence of inflammation of the paranasal sinuses for more than 12 weeks with or without treatment. The cornerstone of accurate diagnosis and treatment of chronic sinusitis is a thorough compilation history and a complete physical examination, including nasal endoscopy and imaging. Before considering surgery the evaluation should clearly indicate that chronic sinusitis is responsible for the patient's constellation of symptoms. Functional endoscopic sinus surgery is considered to be the treatment of choice for chronic sinusitis which does not resolve with conservative medical management. Outstanding short and long-term results have been reported in the literature. In addition, endoscopic sinus surgery significantly improves quality of life.

In this study the effect of functional sinus surgery on nasal mucosa was evaluated using scanning electron microscopy and study of the ultrastructure of cilia, pre and post operatively and its correlation with histopathological examination. The effect on function of the sinus was evaluated by using the mucociliary clearance test, saccharin test, pre and post operatively.

Materials and methods

Twenty patients with chronic sinusitis were selected for this study during a period of 2 years from July 2002 to June 2004. Of these 20 patients 13 were males and 7 were females age ranging from 16–63 years (Mean age: 25.55 yrs). 10 normal patients with no symptoms and signs of inflammation in nose and paranasal sinuses were subjected to Saccharin test and they formed the control group for this

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test. 60% of the control group patients were females and 40% of them were males.

Pre operative Saccharin time on nasal mucosa was recorded in all the patients. All these patients underwent functional endoscopic sinus surgery (FESS). Per operatively a 5×5 mm mucosal specimen from supero-lateral wall and ostium of the maxillary sinus were taken on both sides for scanning electron microscopic study and histopathological examination. Repeat saccharin test and nasal mucosa samples were taken at time of post operative review at 6 weeks. All the above observations were analyzed.

The ultrastructure of the cilia was studied by the scanning electron microscopy. The nasal mucosa was processed before observing in the scanning electron microscope through the following steps.

Primary fixation with 2.5% Glutaraldehyde with buffering done at pH7.4, washing with 0.1 Monophosphate buffer 3 changes of 15 minutes each at 4°C, Post fixation with 1% Osmium sulphate (OS04) for 2 hrs at 4°C, followed again by Washing with 0.1 Monophosphate buffer 3 changes of 15 minutes each at 4°C. Sequential Dehydration initially with 30% Acetone –15 min at 4°C, followed by 50% Acetone –15 min at 4°C, 70% Acetone –15 min at 4°C, 80% Acetone –15 min at 4°C, 90% Acetone –15 min at 4°C, 95% Acetone –15 min at 4°C, 100% Acetone –30 min at 4°C.

Critical point drying using Liquid CO₂ at its critical point 31.5°C at 1100 PSI was done following which the sample mounted on Aluminium stubs. Sample was divided into 2 pieces and one was reversed for examination. Sputter coating was done using Gold. 35 nm thick film coating. The samples were observed in Scanning electron microscopy.

To study the number, density and the regeneration potential of the cilia the parameter of ciliary area is used. This term designates the area occupied by the cilia on the surface of epithelium and was used by Guo et al., in their quantitative study on the cilia of the paranasal sinuses [5].

Results of the study were analyzed on the basis of the outcome of Saccharin test, histopathological examination of goblet cells. Oedema, squamous cell differentiation, basement membrane thickening, vascular congestion, inflammatory cells, fibrosis, ulceration. Sub mucosal gland hyperplasia, Polyp formation and Electron microscopic study of Ultra structure of cilia and Ciliary orientation pre and post operatively.

Patients of age 15–65 yrs from both sexes were included in the study. The inclusion of patients in this study was based on the presence of at least two cardinal symptoms, diagnostic endoscopic findings and computerized tomographic findings of paranasal sinuses. The symptoms included recurrent episodes of headache, nasal obstruction, nasal discharge, post nasal drip. The Diagnostic nasal endoscopic

findings included oedematous or congested mucosa, narrow middle meatus, narrowed osteomeatal complex (OMC), deviated nasal septum, presence of mucopus in middle meatus and anatomic anomalies in middle meatus. The features in CT PNS were mucosal thickening in maxillary sinuses, presence of maxillary polyp, and presence of fluid level, presence of radiological changes in ethmoid or frontal sinuses in addition to maxillary sinuses. narrow OMC and turbinate hypertrophy.

Patients with disorders such as nasal polyps, fungal disease in nose & paranasal sinuses, malignancy, general systemic disorders – Hypertension, Diabetes mellitus, Tuberculosis, previous sinus surgeries, use of nasal steroids pre operatively, smokers except those who have stopped smoking 6 months before study, ciliary disorders Immotile cilia and Kartagener's syndrome were excluded from the study.

Results and observations

Saccharin test timing

The mean pre operative Saccharin test time was found to be 16.20 minutes with post operative mean value of 10.30 minutes. This proved to be highly significant in patient outcome p value : $P < 0.001$ SS (Table 1)

Histopathological changes

Semiquantitative grading was done in comparison with normal mucosal histology. (Table 2 and Table 3)

Table 1 Saccharin test timings

	Mean	n	Std. deviation	Std. error mean
Pre-op time (min)	16.20	20	6.33	1.42
Post-op time (min)	10.30	20	3.71	0.83

$P < 0.001$ SS

Table 2 Semiquantitative grading for histopathological examination

Mucosal histology	Grading
Normal	0
Mild changes	I
Moderate changes	II
Moderately severe changes	III
Severe changes	IV

Table 3 Histopathological observations pre and post operation

HPE	Pre op grade 0/I/II/III/IV	Post op grade 0/I/II/III/IV	P value
Polyp formation	2/4/9/5/0	16/4/0/0/0	>0.05 NS
Goblet cells	0/6/7/4/3	14/6/0/0/0	<0.001 SS
Squamous cell diff.	4/10/4/2/0	18/2/0/0/0	>0.05 NS
Ulceration	1/5/9/5/0	17/3/0/0/0	<0.001 SS
BM thickening	9/9/2/0/0	18/2/0/0/0	<0.001 SS
Oedema	0/1/10/7/2	16/4/0/0/0	<0.01 SS
Submucosal gland hyp	0/0/8/8/4	16/4/0/0/0	<0.05 NS
Vascular congestion	0/1/10/8/1	16/4/0/0/0	<0.05 SS
Fibrosis	0/8/11/1/0	17/3/0/0/0	<0.05 SS
Inflammatory cells	0/0/3/11/6	15/5/0/0/0	<0.01 SS

Polypoid mucosa

Out of 20 cases, 9 cases had moderate polypoidal changes, and 8 of those cases reverted back to normal while only one case had mild changes at the time of review. 5 cases had moderately severe changes out of which 2 reverted to normal and 3 still had mild changes. In remaining 6 cases, 4 had mild changes which completely reverted to normal.

Goblet cells

Goblet cells were present in moderately severe quantity in 4 cases which reverted back to normal in only one case and to mild in 3 cases. It was severe in 3 cases; all of them reverted to mild at the time of review.

Squamous cell differentiation

4 cases had squamous metaplasia of moderate variety out of which 3 returned to normal and one to mild variety. Pre operatively 2 cases had moderately severe differentiation

among which one reverted to normal and one to mild variety.

Ulceration

5 of the 20 cases had moderately severe ulceration out of which 3 reverted to mild ulceration and 2 became normal. (Fig. 1)

Basement membrane thickening:

Preoperatively 2 cases had moderate basement membrane thickening. Both of these cases reverted back to mild at the time of review. (Fig. 2)

Oedema

2 cases had severe oedema preoperatively which at review had mild changes. 2 of the cases had moderately severe edema out of which 2 had mild changes post operatively.

Fig. 1 Ulceration marked in pre op histopathological examination

Fig. 2 Histopathological picture showing basement membrane thickening pre operatively

Scanning electron microscopic findings

The ultra structure of the cilia was studied by using scanning electron microscopy, Philips FEI Quanta 200, which works in low vacuum there by reducing the strain on the specimen. This reduces the chance of ciliary disorientation because of the electron microscope. The preoperative specimen and the post operative specimens were analyzed.

The study done by Halma et al,¹ in 1990 was used as the standard of normal ultra structure and density of the cilia. The pre operative and post operative scanning electron microscopic pictures of the present study was compared by its gross appearance to that of Halma et.al, [1] by a senior pathologist.

The surface of the mucosa was scanned manually for ciliary area.

The preoperative specimens showed a marked decrease in the ciliary area in addition to ciliary destruction and disorientation. There was lot of mucus globules on the ciliary surface. However the density and the exact count could not

Inflammatory cells

Mag 3000 WD 10.5 um HV 2.0 kV Cw 1.0 Spot 3.0 Vacuum Low/Down XMM

Fig. 4 Post-operative normal epithelium 6 weeks

be established because of lack of software image analysis. Post operatively there was a considerable increase in the ciliary area when compared to the preoperative analysis. The findings were near normal / normal in gross appearance in comparison to the work of Halma et al [1]. The ciliary surface had less number of globules and the ciliary orientation was near normal.

Observations

The mucociliary transport mechanism of the sinus mucosa improved 6 weeks following surgery. Histopathologically there was evidence of improvement in the ciliary population and decrease in inflammation, ulceration, fibrosis, vascular congestion, oedema, squamous cell metaplasia, basement membrane thickening and polypoid formation in comparison to the diseased mucosa sampled preoperatively (Fig. 4). Scanning electron microscopic examination revealed considerable increase in the ciliary area as well as the orientation of cilia (Figs. 5, 6). Subjective improvement 6 weeks following surgery, which was confirmed by decrease in saccharin test time.

Discussion

Sinusitis is defined as inflammation of the paranasal sinuses. It is caused either by bacteria, virus, fungi or allergy. Functional endoscopic sinus surgery is considered to be the treatment of choice for chronic sinusitis which does not resolve by medical treatment.

It has been reported in many studies in the literature [2] that FESS normalises maxillary sinus mucosa macroscopically, based on both endoscopic examination and CT evaluation and several authors have studied the effect of endoscopic sinus surgery on the ultrastructure of the sinus mucosa from the qualitative point of view [4]. However, the effect of FESS on the ciliated epithelium of the maxillary sinus has rarely been quantitatively assessed and reported in the available literature [6].

In the present study chronic sinusitis has been found to be associated with marked ciliary loss in the mucosa of the maxillary sinus, and FESS increased the density of the cilia both in the superolateral wall of the maxillary sinus and in the ostial region 6 weeks after surgery.

Saccharin test performed in our series of 20 cases pre and post operatively showed that the clearance time was significantly decreased in the post operative group ($P < 0.001$ SS) thereby suggesting an improvement of ciliary function.

The results of the light microscopic examination of the specimen showed:

- (1) decrease in edema, inflammatory cells, vascular congestion, ulceration, fibrosis, basement thickening and goblet cells.

- (2) non significant changes in the degree of glandular hyperplasia, squamous cell differentiation and polypoid formation.

In this study a considerable decrease in fibrosis, goblet cell population and basement membrane thickening in the sinus mucosa following FESS were observed. Articles related to the above observation was not reported in the literature so far. The ciliary area is calculated using scanning electron microscopy by studying the surface of relatively large areas of mucosa using software image analysis [3]. In our study a manual gross observatory analysis is done because of lack of software image analysis programme. Hence the exact density and count of the cilia could not be analyzed.

Halma et al. showed the distribution of ciliated cells in the normal human nose and paranasal sinus mucosa. They reported that in all parts of the maxillary sinus the density of the cilia was very high ranging from 91.3 % to 97.7 % except at the ostial region where the density dropped nearly half and was approximately 47% [1]. The present study could not arrive at a percentage because of the reason mentioned earlier.

Although the number of the cilia increases significantly after the surgery, this does not mean that the cilia is functioning normally. The normal physiological activity of the regenerated cilia still needs further study [3]. However, the assessment by saccharin test indirectly corroborates that the ciliary function is near normal / normal following FESS. In the present study the physiological activity of the regenerated cilia is assessed by performing a saccharin test post operatively which showed decreased clearance time and hence improved mucociliary clearance after surgery.

The aim of our study was to assess the functional changes in the nasal mucosa following FESS in the treatment of chronic sinusitis. In the published literature [2] this was always done by patient's symptoms using subjective scores taking preoperative and postoperative CT scans or by studying the effect of FESS on the structure and the ultrastructure of the sinus mucosa. However, the success of FESS should be directed towards the validity of the Messerklinger's theory through two ways, firstly clearing the osteomeatal complex region then by associated with ciliary regeneration and increasing the number of cilia in the sinuses, and secondly by proving that these regenerating ciliary area are functioning properly.

In our study of the ultrastructure of cilia, it has been observed that the relative improvement in symptoms directly correlated with the improved mucosal patterns and decreased saccharin time.

Conclusion

FESS is an effective surgical procedure in chronic sinusitis and improves the overall function of the sinuses.

FESS improved mucociliary clearance was measured 6 weeks post operatively. Histopathology showed a significant improvement post operatively with decrease in inflammation, ulceration, fibrosis, vascular congestion, edema, squamous cell metaplasia and basement thickening. Ultrastructural study revealed that surgery improved the orientation and ciliary area to near normal. Also the saccharin test is a simple, safe and reproducible test that should be used in all patients undergoing FESS to measure post operative functional outcome.

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