Alexandria Journal of Medicine (2016) **52**, 243–250

HOSTED BY

ELSEVIER

Alexandria University Faculty of Medicine

Alexandria Journal of Medicine

http://www.elsevier.com/locate/ajme

Impact of osteitis and biofilm formation and correlation between both in diffuse sinonasal polyposis in Egyptian adults; a prospective clinical and histopathologic study

Ayman Moustafa Al-Madani [a], **Suzan Mohamed Helal** [b], **Hoda Mahmoud Khalifa** [c], **Moustafa Mohamed Abdelnaby** [a,*]

[a] *Department of Otorhinolaryngology, Faculty of Medicine, Alexandria University, Egypt*
[b] *Department of Histology and Cell Biology, Faculty of Medicine, Alexandria University, Egypt*
[c] *Department of Pathology, Faculty of Medicine, Alexandria University, Egypt*

Received 7 July 2015; accepted 17 September 2015
Available online 20 October 2015

KEYWORDS

Chronic rhinosinusitis;
Diffuse sinonasal polyposis;
Biofilm;
Osteitis

Abstract *Background:* The pathogenesis of diffuse sinonasal polyposis is still not completely established, possible explanations are osteitis, aeroallergens, fungal sinusitis and biofilms. There are no reports in Egypt about osteitis and biofilms in those patients.
Purpose: To study the incidence and impact of osteitis and biofilms in Egyptian patients on diffuse sinonasal polyposis patients.
Patients and methods: Fifty patients (22 males, mean age of 30.68 ± 7.24 years) submitted to surgery for diffuse sinonasal polyposis. Computerized scan on sinuses ordered and scored by Lund–Mackay staging protocol, severity of Osteitis using the Global Osteitis Scoring Scale. Tissue samples were taken from diseased sinuses to be analyzed histopathologically for osteitis, and with scanning electron microscopy to detect bacterial biofilms. Another ten patients as a control scheduled for septoplasty or turbinectomy with no evidence of sinusitis, and tissue specimens were obtained 1 cm behind the anterior end of inferior turbinate and processed in the same manner for biofilm comparison.
Study design: Contemporary prospective cross-sectional cohort study.
Results: In 70% (35/50) of the polyposis patients, histopathology was positive for osteitis. Biofilms were detected by electron microscope in 39 (78%). Two of controls (20%) were biofilm positive with a significant difference ($p = 0.035$). The mean Lund–Mackay was 19.08 ± 3.67 and mean osteitis score was 18.68 ± 11.99. There was a significant correlation between Lund–Mackay and osteitis score ($p < 0.001$) and between both and histopathologically proven osteitis ($p = 0.049$), biofilms ($p = 0.005$) and postoperative endoscopic healing ($p = 0.046$) where increased soft tissue disease and osteitis and biofilm were associated with bad healing and vice versa.

* Corresponding author.
E-mail address: mostafanaby@gmail.com (M.M. Abdelnaby).
Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2015.09.006

Conclusion: Osteitis and bacterial biofilms underlie the majority of Polypoidal chronic rhinosinusitis and both correlated significantly. Scanning electron microscope is a good tool for detecting bacterial biofilms. Sinus surgery with surgical ventilation, mechanical disruption of biofilms and osteitis is a mandatory therapeutic choice with prolonged treatment with antibiotics and nasal wash.

1. Introduction

Rhinosinusitis is classified according to time into acute cases (lasting up to 12 weeks), subacute (4–12 weeks), chronic (lasting over 12 weeks) and recurrent acute. Chronic rhinosinusitis includes a group without nasal polyps and another with nasal polyps i.e. diffuse sinonasal polyposis (DSNP).[1] DSNP prevalence varies from 1% to 5% and is one of the common complaints in medical visits, and one of the main reasons for antibiotic prescriptions and leave of work.[2]

Diffuse sinonasal polyposis represents a chronic inflammatory condition of unknown definitive etiology till now. It is often associated with systemic diseases and is characterized by nasal obstruction, reduction in sense of smell, recurrent infection, and impaired quality of life. Several factors have been raised to explain the pathophysiology of DSNP as immunological defects, intrinsic airway factors, autonomic imbalance, abnormal transepithelial ion transport, mucopolysaccharide abnormality, enzyme abnormality, allergic and nonallergic rhinitis, *Staphylococcus aureus* superantigen, fungal colonization that induces and maintains eosinophilic inflammation, aspirin hypersensitivity and persistent insult by biofilms and/or osteitis.[3]

The initial traditional approach is medical management. Medical therapy consists of administration of intranasal steroids or a short course of systemic steroids. Other medical treatments considered are use of antibiotics, leukotriene modifiers, and acetylsalicylic acid avoidance.[4]

Surgical removal is performed for non-responders to medical management. The purpose of surgery is to restore the nasal physiology by making the nose mechanically free from nasal polyps and allowing drainage of the infected sinuses and allowing drug delivery to the sinuses. Prolonged medical therapy after surgery is essential for preventing recurrence.[5]

Biofilm consists of grouped microorganism cells anchored irreversibly to a live or inert surface, wrapped in a self-produced extracellular polymer matrix consisting mostly of polysaccharides, which comprises over 90% of the biofilm mass.[6] This condition makes biofilms highly resistant to changes in pH, temperature, and antibiotic action, which possibly explains persistent chronic infections that resist clinical therapy, such as DSNP.[6]

Biofilms have also been demonstrated by scanning electron microscopy (EM) in cholesteatomas, chronic tonsillitis, adenoids of patients with chronic sinusitis, and infections associated with biomaterials such as voice prostheses.[7]

Several studies confirmed the presence of biofilms on the mucosa of patients with chronic rhinosinusitis, which could explain why such patients improved after a course of antibiotics and relapsed after stopping medications.[8] Other studies applying transmission EM and confocal laser microscopy with fluorescence in situ hybridization have confirmed the presence of bacteria inside biofilms.[9] All current biofilm diagnostic modalities require invasive mucosal biopsies, which limit their use to the operating theater.[10]

Chronic rhinosinusitis (CRS) with Osteitis is often associated with recalcitrant disease. The osteitic bones potentially serve as a nidus for inflammation and may explain failures of typical medical and surgical treatment. Osteitis is more associated with previous surgery and the incidence increases with increasing number of previous operations.[11] However, non-operated patients also experience osteitis with an incidence of 5–33% and thus mucosal loss from surgery is not the sole answer to the origins and implication of osteitis.[12] Patients undergoing revision surgery, mucosal eosinophilia and higher serum eosinophils recorded higher osteitis scores.[13]

The significance of osteitis in the management of recalcitrant chronic rhinosinusitis has yet to be clearly understood and clinical outcome data for these patients are lacking. No papers have been published in Egypt about impact of osteitis and biofilms and correlation between both in DSNP or chronic rhinosinusitis. The purpose of this study was to show the incidence of biofilms and/or osteitis in DSNP and its impact on patient's symptoms and post-operative course, also to correlate between osteitis and biofilm presence in DSNP patients.

2. Patients

This was a prospective cohort cross-sectional study. The study group included fifty patients (22 males, 28 females with mean age of 30.68 ± 7.24 years) undergoing FESS for DSNP that did not respond to medical therapy.

Nonresponders are the group of patients who did not respond adequately to medical treatment in the form of short course corticosteroids (15 days), intranasal corticosteroids spray and nasal wash and antibiotics for a period of three months while still having the same symptoms and signs of Polypoidal CRS.[7,30]

Chronic polypoidal rhinosinusitis was defined based on clinical, CT and endoscopic criteria, as follows: a clinical history with two or more of the following symptoms lasting over 12 weeks, one of the symptoms being any of the first two of nasal block or congestion, anterior nasal discharge or posnasal drip, facial pain or sense of pressure, and decreased or absent olfaction; endoscopy revealing bilateral nasal polyps.[1,5]

Those patients with non-polypoidal CRS or with systemic illness were excluded.

The controls were ten patients scheduled for septoplasty for nasal obstruction and/or inferior turbinate reduction with no evidence or history of sinusitis or polyposis, neither clinically nor radiologically.

A full informed consent was signed from all participants and this study was formally approved by the Ethics Committee

of the Faculty of Medicine, Alexandria University with the following ID: [IRB No. 00007555-FWA No. 0015712, June 2013].

3. Methods

All patients were subjected to the following. (1) Full History taking about DSNP including history of bronchial asthma, allergic rhinitis, aspirin intolerance, previous FESS, topical and systemic steroid treatment and systemic antimicrobial therapy, smoking, family history of polyposis and systemic diseases. Subjective assessment of symptoms[2]:

Major symptoms:

- Facial pain\pressure (not considered if no other CRS complaints present)
- Facial congestion\fullness
- Nasal obstruction\blockage
- Nasal discharge\purulence\discolored postnasal discharge
- Hyposmia\anosmia
- Purulence of nasal cavity during examination

Minor symptoms:

Headache, fever, halitosis, fatigue, dental pain, cough and ear pain\pressure fullness.

Symptoms were scored by the visual analogue scale (0–4 scale) for the major and minor symptoms (0 = absent, 1 = mild, 2 = moderate, 3 = moderately severe, 4 = severe).

(2) Complete general Otorhinolarnygoscopic examination.

(3) Nasal endoscopy and reporting the extent of polyposis by using Johansen endoscopic grading system.[3]

(4) Multislice CT scan with no contrast on the nose and paranasal sinuses; coronal, axial, and sagittal CT scans were examined and scored by the Lund–Mackay staging protocol (maximum score is 24) and Global Osteitis Scoring scale.[14] (Table 1)

(5) Complete blood count to record eosinophilia and for preparation for surgery.

(6) During FESS a tissue biopsy was taken from the ethmoid polypoidal tissue and divided into two samples as follows:

Table 1 Determination of the severity of Osteitis in patients with chronic rhinosinusitis using a new Global Osteitis Scoring Scale.

Score	Global Osteitis Scoring Scale
1	Less than 50% of sinus walls involved less than 3 mm thick
2	Less than 50% of sinus walls involved 3–5 mm thick
3	Less than 50% of sinus walls involved more than 5 mm thick or more than 50% less than 3 mm thick
4	More than 50% of sinus walls involved 3–5 mm thick
5	More than 50% of sinus walls involved more than 5 mm thick

The maximum thickness from each sinus wall is measured either by the computer program on the computerized scan or from the scale on the side of each cut. Each sinus to be given 0–5 score of 10 paranasal sinuses, 2 maxillary, 2 anterior ethmoid, 2 posterior ethmoid, 2 frontal, 2 sphenoid. Total score from 0 to 50.
Non-significant less than 5, mild 5–20, moderate 21–35, severe more than 35.

1. Histopathology for detection of osteitis: The samples of tissue biopsy were fixed in 10% formol saline and sent to the laboratory then prepared with xylon; Paraffin blocks will be prepared and 5μ sections will be stained using routine hematoxylin and eosin (H&E) stain. Bony biopsy was processed in acids then after softening and decalcification, the bony specimen was processed as for soft tissue.
2. Biofilm detection by scanning EM: The specimens were examined and photographed with JEOL, JSM-53009 scanning electron microscope in EM unit, Faculty of Science, Alexandria University. All specimens were prepared for SEM using the following techniques. Tissue was initially fixed for 2 h in 2.5% glutaraldehyde in phosphate-buffered saline (PBS, pH 7.4) at 4–8 °C. Two rinses of 15 min each were then carried out using PBS. Next, the specimens were fixed with 1% osmium tetroxide for 1 h. They were then dehydrated through a graded ethanol series as follows: 50% for 15 min, 70% for 15 min, 80% for 15 min, 90% for 15 min, and 100% twice for 15 min each time. The tissue was immersed in 100% acetone for 15 min and washed in 100% isoamyl acetate for 15 min, followed by critical point drying. Finally, specimens were mounted on metal stubs and subsequently sputter coated with gold preparation for imaging.

Structures categorized by water channels, 3D structure, and matrix set in spherical or elliptical bodies were identified as evidence of biofilms. It differs from viscous mucus, the latter is a flat blanket, under which sometimes the comparative orderly cilia could be seen, and irregular foreign granule might be found. The entire area of each specimen was scanned for the presence of biofilm structures. Images were taken at various angles to effectively display the specimens and to minimize errors and artifacts.

In the control group: Tissue specimens of approximately 0.5 cm^3 were obtained 1 cm behind the anterior end of the inferior turbinate and processed in the same manner for detection of biofilm on the surface of mucosa.

(7) Postoperative follow-up: The patients were followed for subjective satisfaction and objective healing of the cavity clinically by endoscopy after surgery. A well healed cavity had healthy mucosal lining with no evidence of inflammation, mucosal swelling, polyposis or scarring.[15]

Data were analyzed using the Statistical Package for Social Sciences (SPSS ver. 20, Chicago, IL, USA). The data were reported as mean and standard deviation. *T*-tests and chi-square tests were used to compare differences in means and proportions where appropriate. The data were compared between the patients and controls using paired *t*-test. Comparison between two independent changes was done using independent two-sample *t*-test. Statistical significance level was set at 0.05.

4. Results

The patients group included 22 males (44%) and 28 females with age ranged from 14 to 50 years with an average of 30.68 ± 7.24 years. Out of fifty patients, 15 (30%) had a family history of DSNP, 18 (36%) were smokers, 25 (50%) had a history of aspirin intolerance, 29 (58%) had

bronchial asthma, 22 (44%) had eosinophilia in their blood and 17 (34%) had a history of previous FESS.

Regarding the symptoms, ten patients had grade 1, nine patients had grade 2, ten patients had grade 3, and 21 patients had grade 4. The mean VAS was 2.84 ± 1.18. There was a significant relation between the VAS and osteitis proven histologically, where presence of osteitis was associated with a higher VAS ($P < 0.001$) and with biofilm, where presence of biofilm was associated with a higher VAS ($P < 0.001$).

The mean endoscopic polyposis grading for the patients was 4.62 ± 1.52; eight patients had grade 2, four had grade 3, ten had grade 4, five had grade 5 and 23 had grade 6. There was a significant relation between nasal polyposis grading and histopathologically proven osteitis ($p = 0.012$) but not with bacterial biofilm presence ($p = 0.065$) nor post-operative endoscopic healing ($p = 0.445$).

There was a statistically significant relation between eosinophilia and osteitis and bacterial biofilm presence, while there is a insignificant relation between eosinophilia and post-operative healing outcome.

The mean LM staging was 19.08 ± 3.67 and there was a statistically significant correlation between LM staging and the GOSS where increasing the extent and severity of mucosal disease is associated with severe osteitis and vice versa ($p = < 0.001$).

There was a significant relation between LM staging and histopathologically proven osteitis, biofilm and postoperative endoscopic healing where increase of soft tissue disease is associated with bad healing and vice versa. (Tables 2–5)

Radiological features of osteitis were present in 40 cases (80%) Figs. 1–3. The mean GOSS was 18.68 ranging from 3 to 40, in those with histologically proven osteitis the mean GOSS was 20.5 while it was 4 in those without osteitis histologically.

4.1. Histopathologic results

Out of fifty patients, 35 cases (70%) were positive for osteitis (Fig. 4) and 15 cases (30%) were negative. There was a significant relation between osteitis proven histopathologically and the severity of symptoms, nasal polyp grading, eosinophilia, GOSS and LM score. Histopathological evidence of osteitis correlated well with the biofilm detection by SEM in 39 patients (78%), 27 patients were positive for both osteitis and biofilm and 12 patients were negative for both.

Table 3 Relation between histologic Osteitis and Lund–Mackay staging.

	Histopathology osteitis		*t*	*p*
	Negative ($n = 15$)	Positive ($n = 35$)		
Lund–Mackay				
Min.–Max.	12.0–20.0	11.0–24.0	2.733	0.049
Mean ± SD.	17.73 ± 2.96	19.66 ± 3.83		
Median	17.0	21.0		

Table 4 Relation between bacterial biofilm and Lund–Mackay staging.

	Biofilm		*t*	*p*
	Negative ($n = 20$)	Positive ($n = 30$)		
Lund–Mackay				
Min.–Max.	12.0–24.0	11.0–24.0	2.924*	0.005*
Mean ± SD.	17.35 ± 3.01	20.23 ± 3.65		
Median	18.0	20.0		

Table 5 Relation between Postoperative Endoscopic Healing with Lund–Mackay.

	Postoperative Endoscopic Healing		*t*	*p*
	Well ($n = 38$)	Bad ($n = 12$)		
Lund–Mackay				
Min.–Max.	11.0–24.0	16.0–24.0	2.053*	0.046*
Mean ± SD.	18.50 ± 3.73	20.92 ± 2.91		
Median	18.50	21.0		

t: Student's *t*-test.

4.2. Bacterial biofilm

All the samples from the DSNP patients showed abnormal findings on the mucosal surface, with varying degrees of sever-

Table 2 Relation between osteitis score and Lund–Mackay staging.

	Osteitis score				*p*
	Not significant (n = 10)	Mild ($n = 23$)	Moderate ($n = 9$)	Severe ($n = 8$)	
Lund–Mackay					
Min.–Max.	12.0–18.0	11.0–20.0	16.0–20.0	13.0–24.0	< 0.001
Mean ± SD.	15.0 ± 3.22	15.50 ± 4.06	18.0 ± 1.74	19.63 ± 4.14	
Median	15.0	15.0	18.0	22.0	

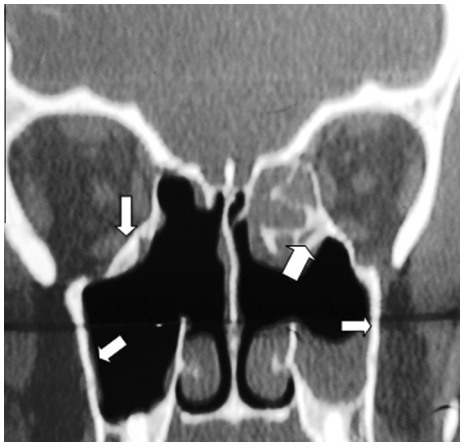

Figure 1 Coronal CT scan showing revision case with severe osteitis of all sinus walls (arrows).

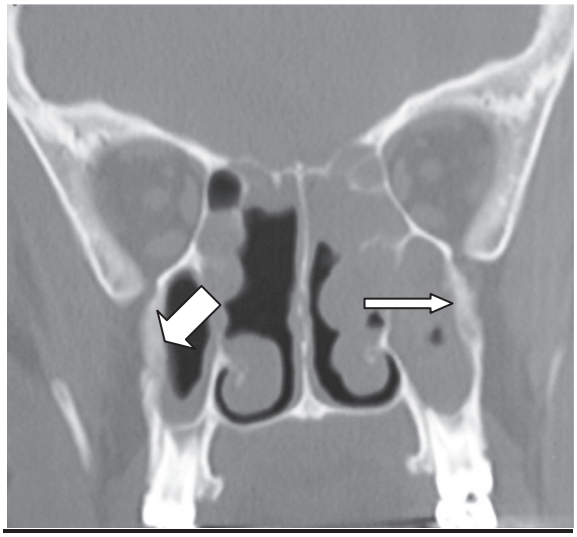

Figure 2 Coronal CT scan showing thickening and rarefaction of lateral maxillary walls (arrows).

ity from disarrayed cilia to complete absence of cilia and goblet cells, even in the absence of biofilm formation, while in the control group, the majority of areas from each specimen showed normal epithelium and cilia (Fig. 5a). Biofilms were detected in 30 (60%) of patients (Fig. 5b, Fig. 6) and it was correlated well with osteitis and postoperative healing outcome (Table 6). Two of the controls (20%) were positive for biofilm with a statistically significant difference between the patients and controls ($p = 0.035$).

5. Discussion

Several mechanisms have been proposed for the pathogenesis of recalcitrant chronic rhinosinusitis specially the Polypoidal group. The polyp formation may be due to allergy, infection, autonomic imbalance, abnormal transepithelial ion transport, mucopolysaccharide abnormality, enzyme

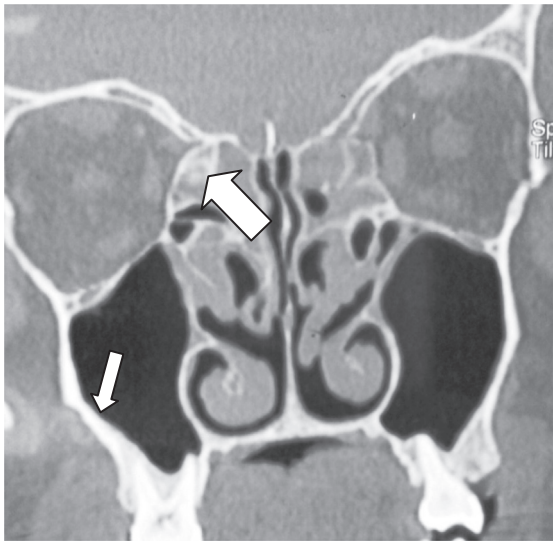

Figure 3 Coronal CT scan showing osteitis of the right maxillary lateral wall (arrow) and ethmoid cell walls (large arrow).

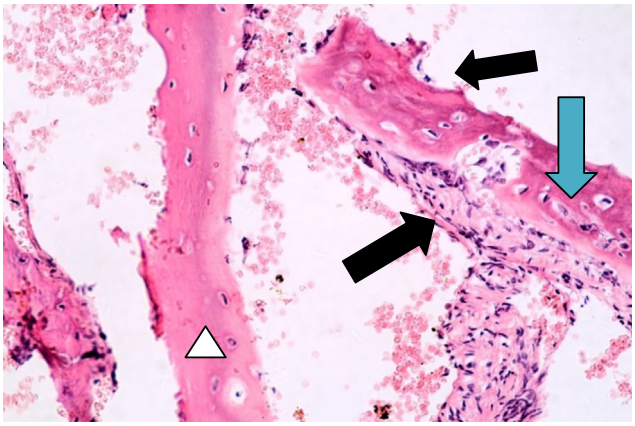

Figure 4 Histologic picture showing fragments of bones interconnected haphazardly with thick, irregular periosteum (black arrow), infiltrated with inflammatory cells. Immature woven bone (blue arrow) and lamellar mature bone (white arrow head). Magnification 200×.

abnormality, mechanical obstruction, epithelial rupture, bacterial biofilm and osteitis.[16]

Nasal polyps are primarily diseases to be managed medically. Although some cases require surgery, with aggressive medical therapy before and after surgery is mandatory. The aim of the treatment is to restore ventilation and sinus drainage as well as to prevent recurrence of the disease.[12]

5.1. DSNP and osteitis impact

The importance of studying the clinical impact of osteitis is perhaps best supported by the relatively high estimated prevalence 36–53% in CRS patients based on either radiographic criteria of bony thickening or pathologic findings. The extent of osteitis has been correlated to objective measures of disease

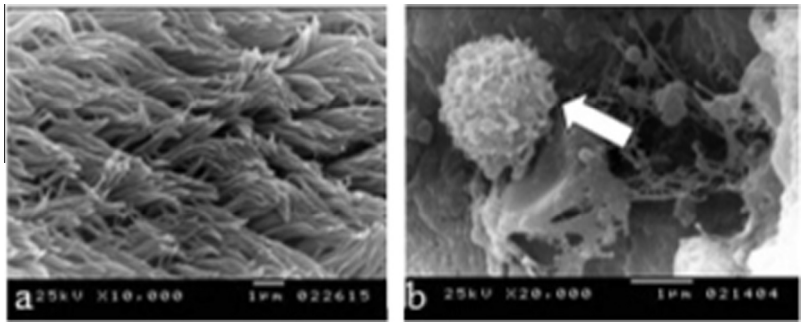

Figure 5 (a) EM picture of normal microvilli sweeping in one direction of mucociliary clearance. (b) EM picture showing bacterial biofilm on the surface of a polyp.

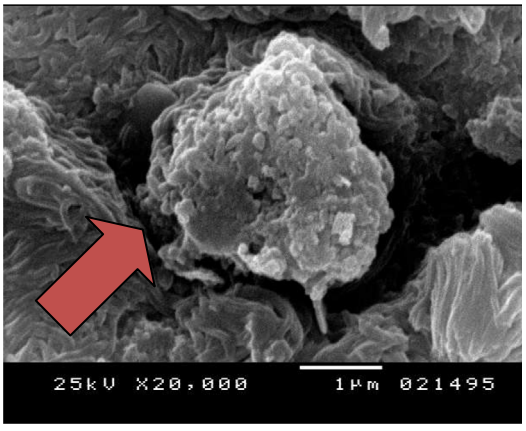

Figure 6 EM picture showing bacterial biofilm on the surface of a polyp.

Table 6 Relation between bacterial biofilm and Histopathology osteitis, Postoperative Endoscopic Healing.

	Biofilm				χ^2	p
	Negative (n = 20)		Positive (n = 30)			
	No.	%	No.	%		
Histopathology osteitis						
Negative	12	60.0	3	10.0	14.286	<0.001
Positive	8	40.0	27	90.0		
Postoperative Endoscopic Healing						
Well	19	95.0	19	63.3	6.597	$^{FE}p = 0.016$
Bad	1	5.0	11	36.7		

χ^2: value for Chi square.
FE: Fisher's exact test.
t: Student's t-test.

severity such as higher Lund–Mackay CT scores, and has a negative impact on quality of life (QOL) outcomes.[17]

The presence of osteitis was associated with a higher VAS than that without and this explains the recalcitrant course of disease in those with osteitis, similarly a significant relation between severity of symptoms and biofilm presence, where biofilm increases the severity of symptoms in CRS and explains the continuous discharge and bad healing postoperatively. This was in agreement with Bhandarkar et al., who found that Osteitis is associated with more recalcitrant CRS. Osteitis is associated with worsened measures of disease severity such as CT, endoscopy polyposis grading and olfactory scores, and affects the degree of improvement in QOL measures after both medical and surgical treatments.[18]

Similarly, Jang et al. discussed 99Tc-MDP bone isotope single photon emission CT in CRS patients to characterize severity of osteitis. Increased isotope uptake was found to correlate to both increased baseline LM scores and worse outcomes following FESS, as assessed by postoperative endoscopy findings of purulence, persistent edema, and recurrence of polyps. Also they found higher baseline CT scores and higher postoperative endoscopy scores in patients with osteitis.[17]

Lee et al. performed a study on 121 patients undergoing FESS for CRS. They found that 53% had pathological evidence of osteitis on histological analysis of surgical specimens.[12]

On the other hand, Sacks et al., found that osteitis is a de novo feature in patients with DSNP even without prior interventions. In these patients, osteitis is associated with high tissue and serum eosinophilia. The study was conducted on unoperated patients with CRS undergoing FESS. Fifty-three patients were included, 42.9% of which had radiologic osteitis. There was no significant association between the presence or severity of osteitis at the time of surgery and symptoms, outcome measures, or endoscopy scores.[19]

Current evidence supporting surgical removal of osteitic bone is anecdotal, suggesting that active inflammation in the underlying bone leads to persistence in overlying mucosal disease which does not resolve until the inflamed bony partitions are removed. FESS plays a critical role in improving treatment outcomes in patients with osteitis.[17]

5.2. DSNP and bacterial biofilm impact

It is now believed that 99% of all bacteria exist in biofilms and only 1% lives in a free-floating or planktonic state at any given time. Recent publications by the Centers for Disease Control and Prevention estimate that at least 65% of all human bacterial infectious processes involve biofilms. Biofilm-positive patients tend to have a greater severity of disease preoperatively and also have persistent and more severe symptoms post-FESS. This study supports the role of biofilms in maintaining the chronic and recalcitrant nature of CRS.[20]

Zhang et al. studied the question how much CRS patients with bacterial biofilms can benefit from FESS. It was found that patients with biofilm-forming bacteria had significantly worse preoperative Sino-Nasal Outcome Test-22 scores compared to those without. Both groups had clinically significant QOL improvement after FESS.[21]

Difficulties in demonstrating biofilms in cultures of patients with CRS may be explained by the presence of bacterial genes that become active in response to specific environmental conditions; in common culture media, the bacteria do not form biofilms and are susceptible to antibiotics.[22]

Biofilms demonstrated by scanning EM in the present study were confirmed with images that are similar to those published in previous studies. Biofilms were identified in 30 (60%) of patients. This highlights the importance of reevaluating the current treatments of CRS, because antibiotics have already been shown to be ineffective against biofilms. Surgical ventilation, mechanical disruption of biofilms and detergents may become a mandatory therapeutic choice. Surgery may be effective because it causes the infected cavity to be ventilated, thus increasing the oxygen tension in the ambience around biofilms.[8]

The reported incidence rates of biofilms in CRS patients range from 25% to 100%, with most studies showing rates of 70–80%.[23] This may be due to different populations, selection of materials for testing which represent only a small fraction of the total sinus mucosa, and also the technique used.

Many studies have correlated the presence of biofilms with a poorer prognosis in patients with CRS.[24] Other studies have tried alternative methods for removing biofilms, such as using children's shampoo.[25] Surgical failure may be accredited to biofilms when these are not eradicated. Biofilm persistence in the folds of edematous chronically inflamed mucosa with absent cilia may lead to rapid reinfection.[17] Further studies, however, are needed to define whether biofilms are the cause or consequence in DSNP patients. Relevant organisms in otorhinolaryngological diseases have been shown to form biofilms, such as Pseudomonas aeruginosa, Haemophilus influenzae, Streptococcus pneumoniae and Staphylococcus aureus.[26]

In fact, the role of bacteria in the pathophysiology of CRS itself is not certain. An inflammatory infiltrate is known to be present in the mucosa of those patients, and serves as the criteria for the mucosal staging systems recommended by Biedlingmaier and others. The release of inflammatory mediators, particularly those of the arachidonic acid pathway, has been postulated by many studies to be the stimulus for bone remodeling in sinusitis.[11,13]

In the control group biopsy taken from the mucosa of the inferior turbinate in cases submitted for septal and/or turbinate surgery and in this group only 2 out of 10 (20%) were positive for biofilm with a statistically significant difference between the study group and control group ($p = 0.035$).

Sanclement et al., reported that with use of strict SEM morphologic criteria as described in the literature as well as by using hundreds of biofilm photographs, examination of the 30 CRS patients' samples revealed evidence of biofilms in 24 (80%) of the subjects. The control cases had healthy appearing cilia and goblet cells without evidence of biofilms.[23]

In the current study, all of the samples from the CRS group showed abnormal findings on the mucosal surface, with varying degrees of severity from disarrayed cilia to complete absence of cilia and goblet cells, even in the absence of biofilm formation, while in the control group, the majority of areas from each specimen showed normal epithelium and cilia. Similarly many studies reported also disarrayed cilia and disorganized mucociliary clearance in cases of CRS even in the cases where there were no biofilms detected but none of the healthy control group.[23,27]

In this study, Histopathological evidence of osteitis correlated well with the biofilm detection by the scanning EM in 39 patients out of 50 (78%), 27 patients were positive for both osteitis and biofilm and 12 patients were negative for both. This was in agreement with, Dong et al. who conducted study on 84 CRS patients undergoing FESS and 22 control patients were enrolled in this study. Mucosal and bony samples from ethmoid sinus were obtained for confocal scanning laser microscopy and microscopic examination. A total of 84.8% of the bone underlying mucosa with BBF had some form of osteitis in ethmoid sinus, and approximately 46.4% of CRS patients were from a subgroup with both BBF and osteitis. In his study, the volume of BBF correlated well with severity of osteitis in CRS patients.[28]

5.3. Postoperative healing

Snidvongs et al., defined a well healing cavity after FESS as a patent cavity with healthy mucosa without edema, polyps, purulence, adhesions or synechia. He found a statistically significant association between worse healing and osteitis.[29]

In this study, biofilm and osteitis had a significant relation with postoperative healing where biofilm and osteitis are associated with worse healing than those without. Similarly other studies found that Osteitis and neo-osteogenesis may also affect the success rate after sinus surgery. They studied the correlation between pre-operative bony changes detected in CT scan and postoperative endoscopic signs of healed sinus cavities in 81 patients. Patients with no radiological signs of bony changes showed better healing mucosa compared to those with bony changes.[28]

6. Conclusion

This study evidences the presence of biofilms in DSNP Egyptian patients using scanning EM, showing their 3-D structure, spherical structures surrounded by an amorphous matrix and its water channels. Osteitis of the sinus walls underlies the pathology in the majority of those patients. FESS with surgical ventilation, mechanical disruption of biofilms and osteitis is a mandatory therapeutic choice with continuation of the medical treatment postoperatively. Osteitis and biofilm coexist together so multimodality therapy is required with both intravenous antibiotics and strong jet nasal wash with high concentration of local antibiotics.

Conflict of interest

Authors state that there is no conflict interest statement.

References

1. Meltzer E, Hamilos D, Hadley J, et al. Rhinosinusitis: establishing definitions for clinical research and patient care. *Otolaryngol Head Neck Surg* 2004;**131**(6):S1–S62.

2. Fokkens W, Lund V, Mullol J. European Position Paper on Rhinosinusitis and Nasal Polyps Group. EP3OS: European position paper on rhinosinusitis and nasal polyps. A summary for otorhinolaryngologists. *Rhinology* 2007;**45**(2):97–101.
3. Johansen VL, Illum P, Kristensen S, et al. The effect of Budesonide (Rhinocort®) in the treatment of small and medium sized nasal polyps. *ClinOtolaryngol* 1993;**18**:524–7.
4. Mackay IS, Lund VJ. Imaging and staging. In: Mygind N, Lildholdt T, editors. *Nasal polyposis: an inflammatory disease and its treatment*. Copenhagen: Munksgaard; 1997. p. 137–44.
5. Chen Y, Dales R, Lin M. The epidemiology of chronic rhinosinusitis in Canadians. *Laryngoscope* 2003;**113**:1199–205.
6. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G. The application of biofilm science to the study and control of chronic bacterial infections. *J Clin Invest* 2003;**112**(10):1466–77.
7. Zuliani G, Carron M, Gurrola J, et al. Identification of adenoid biofilms in chronic rhinosinusitis. *Int J PediatrOtorhinolaryngol* 2006;**70**(9):1613–7.
8. Cryer J, Schipor I, Perloff J, Palmer J. Evidence of bacterial biofilms in human chronic sinusitis. *ORL J Otorhinolaryngol Relat Spec* 2004;**66**(3):155–8.
9. Sanderson A, Leid J, Hunsaker D. Bacterial biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis. *Laryngoscope* 2006;**116**(7):1121–6.
10. Sun Y, Zhou B, Wang C, et al. Clinical and histopathologic features of biofilm-associated chronic rhinosinusitis with nasal polyps in Chinese patients. *Eur Arch Otorhinolaryngol* 2012;**13**:155–202.
11. Videler W, Georgalas C, Menger D, Freling N, Drunen C, Fokkens W. Osteitic bone in recalcitrant chronic rhinosinusitis. *Rhinology* 2011;**49**(2):139–45.
12. Lee J, Kennedy D, Palmer J, Feldman M, Chiu A. The incidence of concurrent osteitis in patients with chronic rhinosinusitis: a clinicopathological study. *Am J Rhinol* 2006;**20**:278–82.
13. Bhandarkar N, Mace J, Smith T. The impact of osteitis on disease severity measures and quality of life outcomes in chronic rhinosinusitis. *Rhinology* 2011;**49**(2):139–47.
14. Georgalas C, Videler W, Freling W. Global Osteitis Scoring Scale and chronic rhinosinusitis: a marker of revision surgery. *Eur Arch Otorhinolaryngol* 2010;**267**(5):721–4.
15. Snidvongs K, McLachlan R, Chin D, Pratt E, Sacks R, Earls P, et al. Osteitic bone: a surrogate marker of eosinophilia in chronic rhinosinusitis. *Rhinology* 2012;**50**(3):299–305.
16. Costerton J, Stewart P, Greenberg E. Bacterial biofilms: a common cause of persistent infections. *Science* 1999;**284**(5418):1318–22.
17. Jang Y, Koo T, Chung S, et al. Bone involvement in chronic rhinosinusitis assessed by 99mTc-MDP bone SPECT. *ClinOtolaryngol* 2002;**27**:156–61.
18. Bhandarkar N, Sautter N, Kennedy D, Smith T. Osteitis in chronic rhinosinusitis: a review of the literature. *Int Forum Allergy Rhinol* 2013 May;**3**(5):355–63.
19. Sacks P, Snidvongs K, Rom D, Earls P, Sacks R, Harvey R. The impact of neo-osteogenesis on disease control in chronic rhinosinusitis after primary surgery. *Int Forum Allergy Rhinol* 2013 Oct;**3**(10):823–7.
20. Głowacki R, Tomaszewski K, Stręk P, et al. The influence of bacterial biofilm on the clinical outcome of chronic rhinosinusitis: a prospective, double-blind, scanning electron microscopy study. *Eur Arch Otorhinolaryngol* 2014 May;**271**(5):1015–21.
21. Zhang Z, Adappa N, Chiu A, et al. Biofilm-forming bacteria and quality of life improvement after sinus surgery. *Int Forum Allergy Rhinol*. 2015.
22. Foreman A, Jervis-Bardy J, Boase S, Tan L, Wormald P. Noninvasive Staphylococcus aureus biofilm determination in chronic rhinosinusitis by detecting the exopolysaccharide matrix component poly-N-acetylglucosamine. *Laryngoscope* 2012;**8**:145–53.
23. Sanclement J, Ramadan H, Thomas J. Chronic rhinosinusitis and biofilms. *Otolaryngol Head Neck Surg* 2005;**132**(3):414–7.
24. Flook E, Kumar B. Is there evidence to link acid reflux with chronic sinusitis or anynasal symptoms? A review of the evidence. *Rhinology* 2011;**49**(1):11–6.
25. Chiu A, Palmer J, Woodworth B, et al. Baby shampoo nasal irrigations for the symptomatic post-functional endoscopic sinus surgery patient. *Am J Rhinol* 2008;**22**(1):34–7.
26. Post J, Stoodley P, Hall-Stoodley L, Ehrlich G. The role of biofilms in otolaryngologic infections. *Curr Opin Otolaryngol Head Neck Surg* 2004;**12**(3):185–90.
27. Psaltis A, Weitzel E, Ha K, Wormald P. The effect of bacterial biofilms on post-sinus surgical outcomes. *Am J Rhinol* 2008;**22**(1):1–6.
28. Dong D, Yulin Z, Xiao W, Hongyan Z, Jia L, Yan X, et al. Correlation between bacterial biofilms and osteitis in patients with chronic rhinosinusitis. *Laryngoscope* 2014 May;**124**(5):1071–7.
29. Snidvongs K, McLachlan R, Sacks R, Earls P, Harvey R. Correlation of the Kennedy Osteitis Score to clinicohistologic features of chronic rhinosinusitis. *Int Forum Allergy Rhinol* 2013;**3**:369–75.
30. Young Lee C, Stow Nicholas W, Zhou Lifeng. Efficacy of medical therapy in treatment of chronic rhinosinusitis. *Allergy Rhinol (Providence)* 2012;**3**(1):e8–e12.