

Considerations regarding the nasopharyngeal bacterial biofilm in pediatric patients

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ABSTRACT. The main target of our study is to correlate the ratio of adenoid mucosa contaminated with bacterial biofilm in pediatric patients suffering of chronic rhinosinusitis (RSC) in comparison to pediatric patients diagnosed with obstructive sleep apnea syndrome (OSAS). We have estimated using s image analysis program the bacterial biofilm from the entire surface of the extracted adenoids, from 5 girls and 15 boys aged between 4 and 10 years; Adenoids extracted from pediatric patients diagnosed with CRS presented bacterial biofilms coating almost the entire mucosa (77.23%), compared to 2.20% of bacterial biofilm coverage, at the pediatric patients with OSAS. The obtained difference is statistically significant. Polyps removed from patients with CSR have most of their mucosal covered with bacterial biofilm in comparison to the group with OSAS. In the nasopharynx of pediatric patients with CSR, bacterial biofilm can play the role of a chronic fountain of pathogens, adenoidectomy explains the symptomatic improvement observed in this group.

KEY WORDS: bacterial biofilm, scanning electron microscopy, adenoids, sleep apnea, chronic rhinosinusitis.

INTRODUCTION

Chronic rhinosinusitis (CRS) in children represents an elaborate disease, with a great reaction on the Romanian economy. ENT specialists from all over the world, think that the secret in treating CRS is a long time antibiotic administration.

Chronic rhinosinusitis is more common in the pediatric population due to an increased frequency of exposure to upper respiratory tract infections in this group of patients.

Bacterial biofilms are highly associated with the chronic infectious processes. The first scientist who described this notion of bacterial biofilm bent to a surface was Antonie van Leeuwenhoek (1632-1723) by the use of a light microscope and he described the dental plaque (Donlan RM, 2002). Several nosocomial infections associated with the usability of different medical devices like tympanic ventilation tubes, urethral catheters, tracheal canulas, orthopedic equipment, artificial heart valves, venous catheters and other are related with the adherence of the bacterial biofilms to their surface (Stewart PS, Costerton JW, 2001).

The life of bacteria inside the biofilms can be divided into 3 parts: the attachment, the growth, and the detachment. (Ramadan HHSanclement JATHomas JG, 2005) In the first phase, the bacteria irreversibly attach to a surface. As the bacterial cells start to divide, the exopolysaccharide pattern is formed and the biofilm starts to form many towers and water channels through the matrix; these water channels play

an important role regarding the waste disposal and help set the pH. The individual bacteria start to spout from the biofilm to conquer new surfaces. The chemical signaling, quorum sensing, permits for cell-cell communication inside the biofilm. Antibiotic therapy may reverse symptomatology caused by outpouring of the planktonic bacteria; anyway unless the entirely colonized surface is removed, the infection will reappear. (Costerton JW, Stewart PS, Greenberg EP, 1999). Infections related to bacterial biofilms are highly difficult to cure due to the secondary resistance to the antimicrobial therapy. The National Institutes of Health, states that even more than 60% from all human bacterial infections implicate bacterial biofilms. (Lewis K, 2001)

In patients suffering of CRS, we do not encounter the same kind of organisms that are responsible for acute sinusitis. Some alternatives to oral antibiotic therapy regarding CRS, include: adenoidectomy, functional endoscopic sinus surgery, long-term parenteral antibiotic treatment. Recent study shows that anaerob bacteria were cultivated from the sinuses of pediatric patients with CRS at a rate between 2% and 100%. Adappa and Coticchia (Adappa ND, Coticchia JM, 2006) accomplished in their study a 91% success percent regarding the therapy of 22 pediatric patients with CRS during 5 weeks with intravenous antibiotic treatment with simultaneous adenoid removal. In another study made by Vandenberg and Heatley (Vandenberg SJ, Heatley DG, 1997), obtained the following results:

58% of the patients- children had almost complete resolution of CRS symptoms after adenoidectomy. Late discovering show that adenoidectomy alone may have positive effects on the patients suffering of CRS. All these studies suggest that adenoidectomy with or without FESS significantly improves rhinosinusal symptoms.

A new idea regarding the bacterial biofilm was born when Characklis(1970) noted its resistance to disinfectants. He stated that bacteria no longer exist singularly as planktonic particles but rather in organized systems even in a human host.(Characklis WG, 1973) These systems of organization are well adapted for rough conditions of environmental stress.

It has been stated that, almost 99% of the bacterial population exist in the form of bacterial biofilm, very difficult to eradicate. Bacterial biofilm formation on adenoids represents a system of propagation for the chronic infection to the rhinosinusal mucosa.

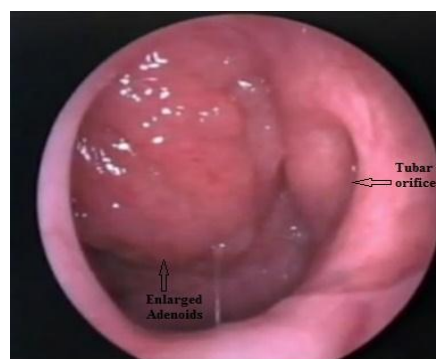
To prove a new epitome in the genesis of CRS, our team of scientists purposed to show the presence of bacterial biofilm on the surface of the adenoids extracted from pediatric patients diagnosed with CRS in comparison to pediatric patients diagnosed only with obstructive sleep apnea syndrome (OSAS). To realize this, we used scanning electron microscopy (SEM) for bacterial biofilm detection on the surface of the tissue samples. Even though we applied different therapy protocols in the try to treat our pediatric patients suffering from CRS, we came across multiple therapy downfalls; that is why we postulate that bacterial biofilm formation could have a central part in the origin and recurrence of CRS.

MATERIALS AND METHODS

We enrolled in our study 20 pediatric patients, 5 girls and 15 boys aged between 4 years and 10 years, suffering from CRS and OSA. We divided the patients in two groups: the CRS group, and the OSASgroup. All patient parents/caregivers signed a written informed consent regarding the agreement to undergo the surgical intervention. All patients parents/caregivers were fully informed before deciding to undergo the surgical intervention. All children underwent full ENT(ear, nose, throught) exam, bloodwork, pulmonary radiography, allergy tests to all drugs that followed to be administered to them, and a cardiological consult with ecg and obtained cardiologic approval for general anesthesia. All examinations, ENT endoscopy, surgical interventions, and drug administrations were documented in the medical records of the patients. ENT endoscopy was performed with 0° Storz rigid 4 mm endoscopes, Storz telecam SL II Camera and a Storz Halogen 250 twin light source.

We defined CRS as an infectious state, lasting longer than 3 months, that failed long term course of oral antibiotherapy- for at least 5 weeks. We only included in this group pediatric patients with CRS and documented hipertrophic adenoids, diagnosed using rigid nasal and rhinopharyngeal endoscopy.(Figure A)

Figure A- Enlarged adenoids- endoscopic image

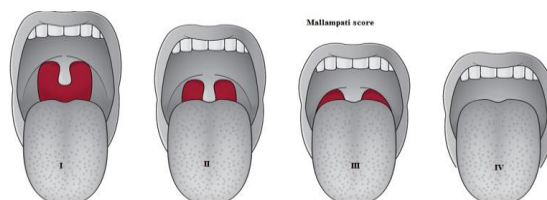


From a clinical point of view the patients from the CRS group suffered at least two of the following symptoms: nasal obstruction, hyposmia/ anosmia, purulent nasal discharge, anterior/ posterior nasal drip, facial fullness.

We defined the OSAS as an endoscopic documented choanal obstruction over $\frac{3}{4}$, and a Mallampati score higher than 2, and the confirmation from the parents of sleeping disorders and snoring for a period longer than 12 months.(Fig. B)

After all the investigations we included in our study lots 10 patients with CSR and 10 patients with OSAS.

Figure B, Class I Soft palate, uvula, fauces, pillars visible, Class II Soft palate, uvula, fauces visible, Class III Soft palate, base of uvula visible, Class IV Only hard palate visible



All of the 20 patients underwent endoscopic adenoidectomy and where was the case also FESS. The goal of the surgical therapy is to reestablish normal sinus ventilation and to restore the mucociliary clearance.

We have collected the adenoids from all 20 patients included in the study, and sent them for SEM preparation to our microscopy lab.

The fixation for the SEM adenoid specimens was realized in 2.5 % glutaraldehyde in PBS buffer (pH 7.4) for 2 h at 22°C. The probes were post-fixed for 1 h with 1 % osmium tetroxide in PBS at 37 °C. After the fixation, the specimens were dehydrated using graded ethanol series and immersed 5 times in a solution of hexamethyldisilazane for a 10 minute period, and left to dry overnight. Specimens obtained were mounted and silver sputter coated in the terminal preparation.

We detected the bacterial biofilm using a Scanning electron microscope FEI Quanta 250 from

the Vasile Goldis Western University of Arad, Microscopy lab. We measured the bacterial biofilm from the entire surface of the extracted adenoids from the SEM obtained images, from the 20 pediatric patients using Carnoy software.

For feedback we practiced routine control visits at 1, 3 and 6 months from the intervention, with the help of the parents and the family doctors of the pediatric patients.

RESULTS AND DISCUSSIONS

In **Figure C**, we show SEM image of adenoid tissue removed from a patient suffering from CRS. In **Figure D**, we present SEM image of an adenoid surface from a pediatric patient with OSAS. Carnoy software evaluated the bacterial biofilm surface field of every sample examined using SEM. **Table nr.1** shows the obtained percentages of biofilm coverage at each of the 20 patients, demographics and diagnoses.

Table nr.1

Patient number	Age (years)	Sex	Diagnosis	Bacterial Biofilm Coverage (%)
1	4	F	OSAS	1,32
2	6	F	OSAS	2,72
3	4	M	OSAS	0,41
4	7	F	OSAS	4,00
5	8	M	OSAS	2,15
6	5	M	OSAS	0.93
7	8	M	OSAS	2.33
8	4	M	OSAS	0.55
9	7	M	OSAS	3,11
10	13	F	OSAS	4,54
11	6	F	CRS	98,13
12	15	M	CRS	97,44
13	11	M	CRS	89,98
14	4	M	CRS	67,65
15	8	M	CRS	77,05
16	10	M	CRS	88,43
17	9	M	CRS	99,12
18	5	M	CRS	59,99
19	8	M	CRS	87,76
20	10	M	CRS	94,56

Figure C, Typical bacterial biofilm morphology in the adenoid mucosa from a patient documented with
SEM image- Magnification x 2000

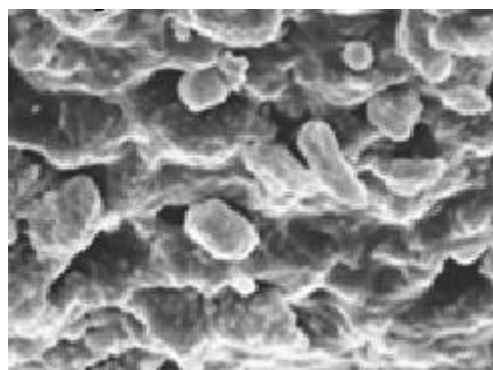


Figure D, Adenoid mucosa from a patient with diagnosed OSAS, with no sign of bacterial biofilm structure.

SEM image- Magnification x 2000

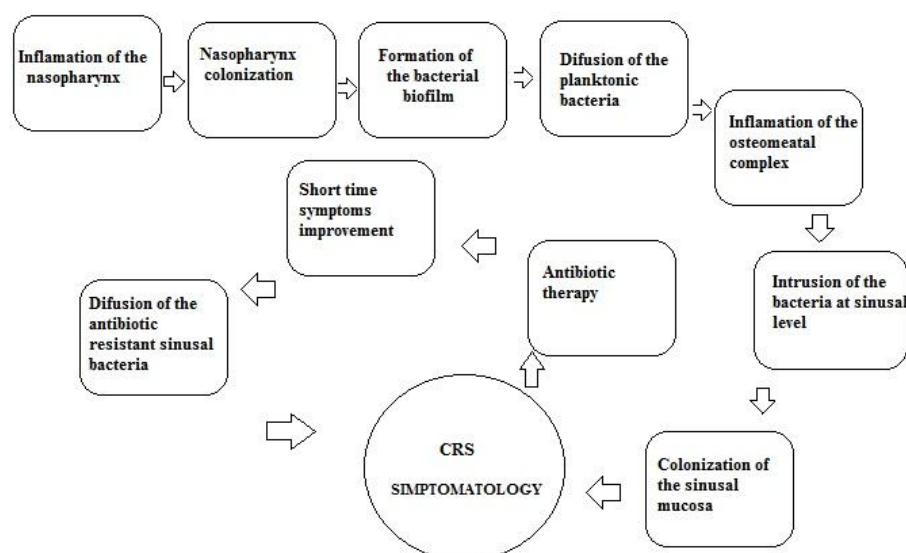


Nasopharyngeal adenoids extracted from the pediatric patients with CRS presented bacterial biofilms covering the entire field of the mucosa with a range of 77.23% of coating.

Nasopharyngeal adenoids extracted from the pediatric patients with OSAS presented a very low coverage of bacterial biofilm at the microscopic examination, with an average of 2.20% of the surface covered with biofilm-like structures. The obtained difference between the 2 groups of pediatric patients is statistically significant.

We believe that the adenoids from the nasopharynx of the pediatric patients with CRS acts as a chronic fountain of pathogens, facilitating the colonization of planktonic organisms, assuring a cycle of reinfection as follows: planktonic bacteria waves, from the nasopharyngeal biofilm, during the breathing cycles, reach into the sinuses producing the colonization and CRS symptoms. After administration of oral antibiotic therapy, all CRS patients show a short time improvement of the symptomatology followed by the quick rebound of the symptoms. This symptomatic rebound that we write about could be due to the diffusion of the antibiotic resistant bacteria from the sinus and adenoids biofilm, that trigger another episode of CRS. **Figure E**

Figure E, Bacterial colonization of the rhinosinusal mucosa from the contaminated adenoids.



There are many therapeutical protocols regarding the cure of CRS in children like oral or parental antibiotics, saline rinses, intranasal steroids. In most of the cases, these measures prove to be insufficient and the medical therapy fails.

In our ENT Clinic we practice 2 main surgical protocols, regarding the treatment of CRS as follows: adenoidectomy, sinus lavage, and oral/parenteral antibiotic therapy and adenoidectomy combined with functional endoscopic sinus surgery (FESS). The first protocol assures the débridement of the nasopharyngeal biofilm and modifies the oxygen gradient in the sinus and the antibiotic therapy aims the active bacteria. The second protocol assures the mechanical removal of the paranasal and nasopharyngeal mucosa sinus biofilm contaminated and raises the oxygen pressure inside the sinus.

The ability to acquire images in high magnification, with structural microscopic details, is the main advantage of SEM. Even though artifacts can arise underlying SEM processing, many authors support the fact that the SEM processing does not notably modify the biofilm architecture and the recognition on samples is possible. (Edwin T., Marcelo B.A., James N.P., Noam A.C. and Wilma T.A.-L., 2009)

Other authors proved a prevalence of only 80% of biofilms in nasal mucosa biopsies from CRS patients using SEM. (Sanclement J.A., Webster P., Thomas J., Ramadan H.H., 2005).

In spite of the disadvantages regarding SEM detection of the bacterial biofilm: risk of sample deterioration during fixation and dehydration process, and the long period of time spent in the microscopy lab, we detected the bacterial biofilm in all our CRS patients. Much of the current knowledge about biofilms is due to the advances in imaging studies, especially the SEM.

CONCLUSIONS

SEM examination of the anedoid samples is an efficient method of bacterial biofilm detection on human adenoid tissue

Polyps removed from patients with CSR have almost their entire mucosal covered with biofilm compared to the group with OSAS.

In the nasopharynx of children with CSR, bacterial biofilm can play the role of a pathogen fountain.

Parenteral/oral antibiotic therapy has only a short time effect on the CRS patients, the infection reappears after a short period of symptomatic silence.

All of the CRS group of patients presented significant symptoms improvement after the adenoidectomy and FESS intervention at the routine control visits at 1, 3 and 6 months from the intervention.

Nasopharyngeal polyp extraction and limited FESS surgery represents a pertinent indication in the therapy of CRS nonresponsive to prolonged antibiotic therapy in pediatric patients.

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