Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.234154.2873 Volume 30, Issue 1.7, Oct. 2024, Supplement Issue
Manuscript ID: ZUMJ-2109-2335 (R1) Doi: 10.21608/ZUMJ.2022.88292.2335
ORIGINAL ARTICLE

The Effect of Biofilm on Post-Sinus Surgical Outcome in Chronic Rhinosinusitis

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Submit Date 16-11-2021
Revise Date 03-01-2022
Accept Date 02-02-2022

ABSTRACT

tives: The goal of this study is to evaluate the effect of biofilms on the outcome of functional endoscopic sinus surgery for patients with chronic rhinosinusitis.**Methods:** The study design involved a retrospective analysis of prospectively collected data in a tertiary hospital. The subject population consisted of 25 consecutive patients undergoing ESS for CRS. The diagnosis of CRS was made according to the criteria set out by the Rhinosinusitis Task Force and endorsed by the American Academy of Otolaryngology. Patients included in the study have received standardized preoperative medical therapy. **Results:** We studied 25 patients with a mean age of 34.68±11.44, Regarding sex distribution the majority were male. Age was distributed as 34.68±11.44 with a minimum 18 and a maximum of 11.44 years, Regarding sex distribution males were 64.0% and females 36.0%, and 32.0% of the studied group were smokers. 72.0% of the studied group had co-morbidity the majority were allergic 48.0% and only 32.0% had previous surgery. 72.0% of studied group had nasal block and it was the most prevalent symptom, headache in 56.0%, post nasal drip 36.0%, hyposmia in 48.0%, polyp in 36.0% and facial pain in 56.0%. **Conclusions:** Biofilm-positive -patients tend to have greater severity of the disease preoperatively and continue to have persistent and more severe symptoms post-ESS, with ongoing mucosal inflammation and recurrent infections. This study strengthens the evidence for biofilms' role in recalcitrant CRS. Therapies targeted at removing biofilms may be important in managing recalcitrant CRS.

INTRODUCTION

Biofilms present a new challenging concept in sustaining chronic common antibiotic-resistant ear, nose, and throat infections[1].

Over the past 20 years, a new appreciation has developed regarding how bacteria behave differently once bound to a surface, surface bacteria grow into biofilms which are

colonies of slow-growing bacteria that surround themselves in a coat of glycopolysaccharides called a glycocalyx. The biology of biofilms focuses on their life cycle and interactions with the environment, the life cycle can easily be divided into three parts: attached phase, growth, and detachment [2].

Most biofilm researchers use specialized microscopy to visualize the presence of bacteria, various techniques have been used including scanning electron microscopy, transmission electron microscopy[3], scanning laser confocal microscopy, and three-dimensional resonance imaging[4].

Although cases of paranasal sinusitis with severe suppuration are reported as less frequent, the incidence of biofilms may explain the recalcitrant nature of some forms of chronic rhinosinusitis[5].

Chronic rhinosinusitis can have many independent inciting factors, but the therapy still remains the same: antimicrobial and anti-inflammatory agents combined with surgical ventilation[6].

It is understood that endoscopic sinus surgery will not cure all chronic rhinosinusitis patients, there are still poor outcomes after endoscopic sinus surgery[7].

The development of not only techniques but also modern instruments in endoscopic surgery itself may be an effective method to eradicate biofilms[8].

The visual identification of biofilms represents a surrogate endpoint in proving the existence of biofilms in chronic rhinosinusitis, several hallmark features of biofilms were identified including three-dimensional aggregates of bacteria, and the presence of glycocalyx, and water channels[9].

METHODS

The study design involved a retrospective analysis of prospectively collected data in a tertiary hospital. The subject population consisted of 25 consecutive patients undergoing ESS for CRS. The diagnosis of CRS was made according to the criteria set out by the Rhinosinusitis Task Force and endorsed by the American Academy of Otolaryngology. Patients included in the study have received standardized preoperative medical therapy.

Clinical data, including demographical information, relevant medical and surgical history, asthma, allergy, and smoking status were all recorded. Each patient was asked specifically to indicate the severity (score of 0-5) of the following five sinusitis symptoms: nasal obstruction, rhinorrhoea, postnasal drip, headache/facial pain, and loss of smell. All patients were provided with the patient's questionnaire form (Sinonasal outcome treatment-20), they also underwent preoperative computerized tomography (CT) scanning and were staged and evaluated blindly according to the Lund-McKay CT scoring system. Written informed consent was obtained from all participants, the study was approved by the research ethical committee of the Faculty of Medicine, Zagazig University. The study was done according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

ESS done according to the symptoms of patients and the extent of the disease on CT scan.

The samples are taken intraoperatively; these specimens were acquired from the frontal, sphenoid, anterior, and posterior ethmoid

sinuses, frontal recess, middle nasal concha, and middle nasal meatus. The harvested tissue placed immediately in 2.5% glutaraldehyde in cacodylic acid (0.1 mol/L) titrated to a Ph of 7.2.

The specimens are dehydrated in serial solutions of ethanol (60-100%) for 15 minutes each, with a second soak in 100% ethanol. The specimens were then attached to a copper specimen container with carton tape. Each specimen was subjected to carbon dioxide critical point drying sputter coated with 60/40 gold-palladium then visually inspected with electron microscopy.

Several areas of the specimen are systematically scanned. A sample considered to have a biofilm if three criteria be met: presence of bacterial sized and shaped objects, the presence of an amorphous material consistent with glycocalyx around the bacteria, and surface binding.

After the endoscopic sinus surgery done, patients irrigated the nose with normal saline three times a day for 4 weeks. Patients followed up every week in the postoperative month then monthly till the six months, after 6 months the participants are seen in the OPD to complete the questionnaire form again and are assessed endoscopically.

All follow-up and endoscopies were performed by a single observer, who was blinded to the biofilm status of the patient.

The collected data were coded, processed, and analyzed using the SPSS (Statistical Package for Social Sciences) version 15 for Windows® (SPSS Inc, Chicago, IL, USA). Qualitative data was presented as number and percent. Comparison between groups was done by the Chi-Square test. Quantitative data was tested for normality by the Kolmogrov-Smirnov test. Normally distributed data was presented as mean ± SD. $P < 0.05$ was considered to be statistically significant.

RESULTS

We studied 25 patients with a mean age of 34.68±11.44, regard sex distribution the majority were male, Age was distributed as 34.68±11.44 with a minimum of 18 and maximum of 11.44 years, regard sex distribution male was 64.0% and 36.0%, and 32.0% of the studied group were smokers,72.0% of studied group had co-morbidity the majority were allergic 48.0% and only 32.0% had previous surgery, 72.0% of studied group had a nasal block and it was the most prevalent symptom, headache in 56.0%, post nasal drip 36.0%, hyposmia in 48.0%, polyp in 36.0% and facial pain in 56.0%, CT score was distributed as 11.76±3.88 with minimum 4 and maximum 21, the not satisfied group significantly associated with higher CT score also with smoking, history of previous surgery, hyposmia, polyp, facial pain and also with positive biofilm and with a higher grade of SNOT, Positive biofilm group significantly associated with higher CT score with less facial pain, higher grade of SNOT-20 and no satisfaction.

Table 1: Demographic data distribution among studied group (N=25)

		AGE	
Mean± SD		34.68±11.44	
Median (Range)		33.0 (18-60)	
		N	%
Sex	Male	16	64.0
	Female	9	36.0
Smoker	Non	17	68.0
	Smoker	8	32.0
	Total	25	100.0

Table 2: clinical picture distribution among the studied group (N=25)

		N	%
Nose block	-VE	7	28.0
	+VE	18	72.0
Headache	-VE	11	44.0
	+VE	14	56.0
Post nasal drip	-VE	16	64.0
	+VE	9	36.0
Hyposmia	-VE	13	52.0
	+VE	12	48.0
Polyp	-VE	16	64.0
	+VE	9	36.0
Facial pain	-VE	11	44.0
	+VE	14	56.0
	Total	25	100.0

Table 3: biofilm distribution among studied group (N=25)

		N	%
Biofilm	-VE	9	36.0
	+VE	16	64.0
	Total	25	100.0

64.0% were positive regard biofilm

Table 4: Satisfaction distribution among studied group (N=25)

		N	%
Satisfaction	Not	17	68.0
	Satisfied	8	32.0
	Total	25	100.0

32.0% were satsatisfied and 68.0% were not satisfied

Table 5: relation between Biofilm and other parameters

			Negative	Positive	t	P
AGE			35.11±12.36	34.43±9.74	0.138	0.891
CT_SCORE			9.44±2.00	13.06±4.62	2.185	0.039*
SEX	Male	N	5	11		
		%	55.6%	68.8%		
	Female	N	4	5	0.43	0.509
		%	44.4%	31.2%		
Smoker	Non	N	8	9		
		%	88.9%	56.2%		
	Smoker	N	1	7	2.82	0.093
		%	11.1%	43.8%		
Co-morbidities	No	N	4	3		
		%	44.4%	18.8%		
	Yes	N	5	13	7.79	0.16
		%	55.6%	81.2%		
Previous surgery	No	N	8	9		
		%	88.9%	56.2%		
	Yes	N	1	7	2.82	0.093
		%	11.1%	43.8%		
Nose block	-VE	N	2	5		
		%	22.2%	31.2%		
	+VE	N	7	11	0.233	0.62
		%	77.8%	68.8%		
Headache	-VE	N	5	6		
		%	55.6%	37.5%		
	+VE	N	4	10	0.76	0.38
		%	44.4%	62.5%		
Post nasal drip	-VE	N	7	9		
		%	77.8%	56.2%		
	+VE	N	2	7	1.15	0.28
		%	22.2%	43.8%		
Hyposomia	-VE	N	7	6		
		%	77.8%	37.5%		
	+VE	N	2	10	3.74	0.053
		%	22.2%	62.5%		
Polyp	-VE	N	8	8		
		%	88.9%	50.0%		
	+VE	N	1	8	3.78	0.052
		%	11.1%	50.0%		
Facial pain	-VE	N	1	10		
		%	11.1%	62.5%		
	+VE	N	8	6	6.17	0.013*
		%	88.9%	37.5%		

			Negative	Positive	t	P
SNOT	I	N	7	1		
		%	77.8%	6.2%		
	III	N	1	11	13.75	0.001**
		%	11.1%	68.8%		
	IV	N	1	4		
		%	11.1%	25.0%		
Satisfaction	Not	N	2	15		
		%	22.2%	93.8%		
	Satisfied	N	7	1	13.54	0.00**
		%	77.8%	6.2%		
Total		N	9	16		
		%	100.0%	100.0%		

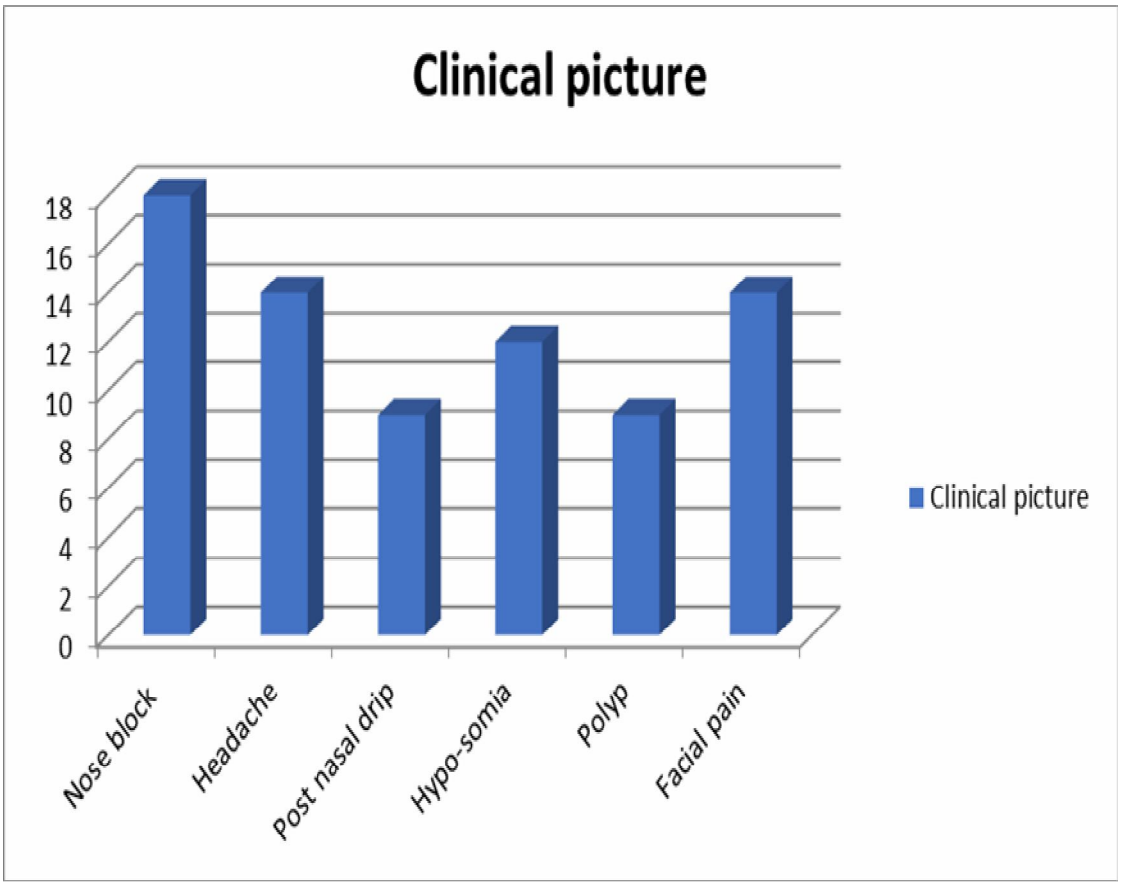

Figure1

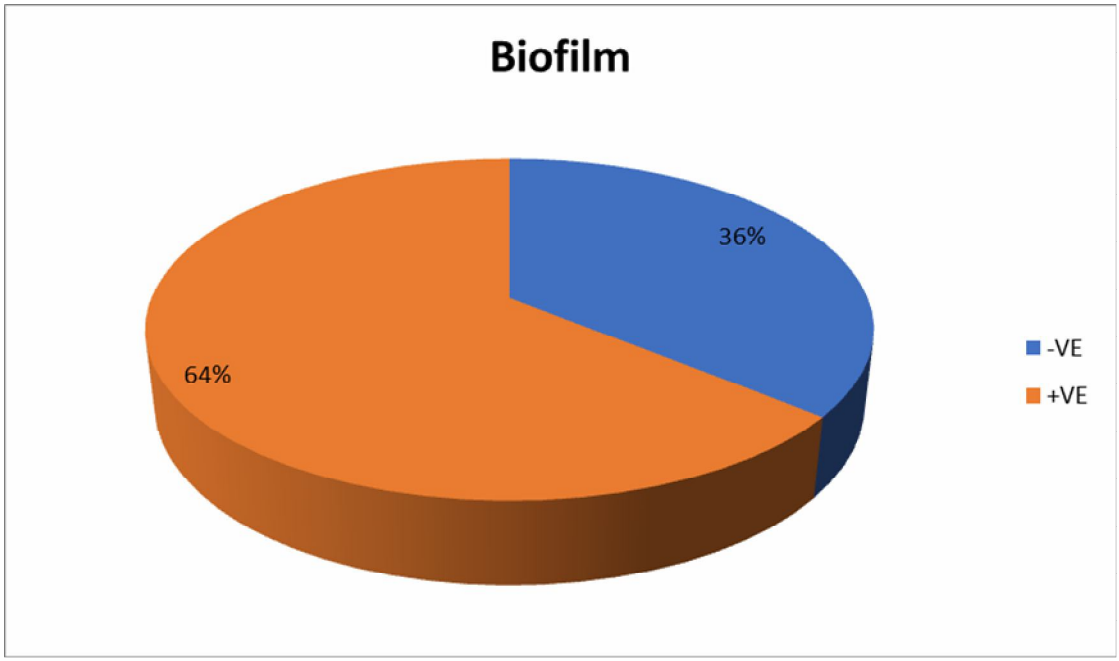

Figure2

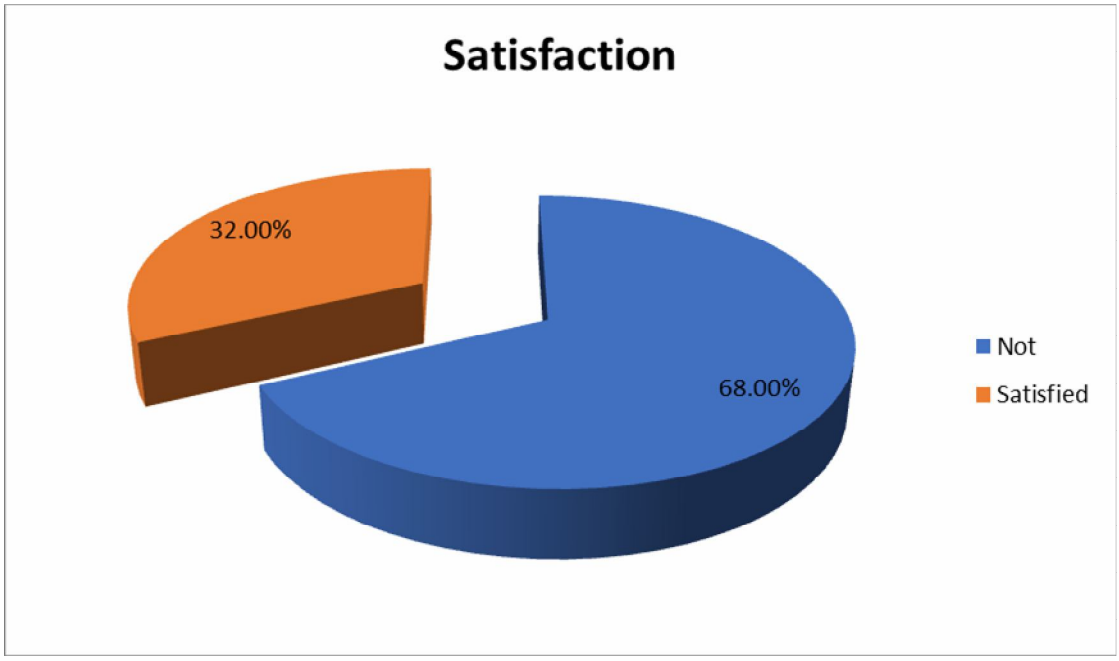

Figure3

DISCUSSION

This study aimed to assess the influence of bacterial biofilms in CRS patients on the clinical outcomes following ESS.

Using scanning electron microscopy, biofilms were shown in 64% of the group studied; patients with positive biofilm were significantly associated with higher CT scan scores and higher grades of SNOT-20 and with no satisfaction.

In the study we noticed that not satisfied group significantly associated with higher CT score also with smoking, history of previous sinus surgery, hyposmia, nasal polyp, facial pain, and also with positive biofilm and with higher grade of SNOT-20.

This prospective, double-blind study has shown that biofilm-positive patients have worse subjective and objective disease scores, have poorer postoperative outcomes and have

a higher risk of disease recurrence when compared with biofilm-negative patients.

Our result agrees with **Psaltis et al.** [10] who found that biofilms indeed may play an active role in perpetuating inflammation in CRS patients and may explain the recurrent and resistant nature of this disease.

This was in agreement with **Singhal et al.** [11] who proved that patients with biofilms have more severe disease preoperatively and persistence of postoperative symptoms, ongoing mucosal inflammation, and infections.

Our result is well matching that patients with biofilm forming bacteria have significantly poor preoperative and postoperative SNOT-20 score and endoscopy score as compared to biofilm-negative patients.

This statistically significant poor post-operative symptom outcome in patients with biofilms indicates that this subgroup of CRS patients continues to have an ongoing relapsing and recalcitrant course. Residual biofilms after surgery may seed the regenerating epithelium and serve as a nidus for further biofilm formation. Bacterial biofilm flora is formed when BF flora secretes extracellular polysaccharide capsule polymers that contain bacteria. Once established, bacteria within biofilm have altered phenotype/genotypes, making them extremely resistant to the host immune system and antibiotics. The biofilm may act as a stimulus for ongoing inflammatory response, as well as releasing planktonic forms of bacteria periodically as a part of its life cycle, leading to acute exacerbations on top of the chronic process. Superantigens secreted by the bacteria can directly stimulate lymphocytes and proinflammatory cells to release inflammatory mediators, resulting in mucosal inflammation.

In addition, biofilms, thanks to their complex structure, can adapt to the surrounding chemical and physical environment. Thus, the bacteria at deeper biofilm layers develop resistance to antibiotics [12]. When pharmacological treatment ends, the bacteria multiplate, and biofilm can be stored in a matter of hours. This shows the need to completely excise the mucosa carrying the biofilm during ESS. Unfortunately, this is not possible as the operator's eye cannot see the exact location and extent of mucosa covered by the biofilm. Thus, the necessity for a thorough combined surgical and pharmacological treatment in the case of biofilm-positive CRS patients.

In contrast to our result, **Zhang et al.** [13] reported that CRS patients with biofilm-forming bacteria demonstrated clinical QOL improvement following FESS, but the degree of improvement decreased over time and became significantly worse than patients without biofilm-forming bacteria by 6 months follow-up.

Following new literature, this study has one potential the use of SEM and not laser scanning confocal microscopy (LSCM). At present, multiple imaging modalities are applied to document the presence of bacterial biofilms. Electron microscopy is the classic approach, which is said to b limited in clinical utility by inherent problems associated with tissue preparation and sampling. The gold standard for bacterial biofilm identification is fluorescence in situ hybridization, **Hogardt et al.** [14]**.** However, **Foremane et al.** [15] recently unveiled an equivalent sensitivity of backlight/LSCM and FISH/LSCM.in comparison to FISH/LSCM, BacLight/LSCM is preferred in bacterial biofilm screening in clinical practice, with the advantage of being rapid, simple, interobserver reliable, and covering all biofilm species. however, we want to defend the use of SEM, as it can clearly distinguish the morphologic characteristics of biofilms. Indeed, it cannot determine the species of bacteria that form the biofilm, but this was not the subject of our study. We used SEM only to determine the presence of or lack of biofilms.

CONCLUSION

Biofilm-positive -patients tend to have greater severity of the disease preoperatively and continue to have persistent and more

severe symptoms post-ESS, with ongoing mucosal inflammation and recurrent infections. This study strengthens the evidence for biofilms' role in recalcitrant CRS. Therapies targeted at removing biofilms may be important in managing recalcitrant CRS.

CONFLICT OF INTEREST

The authors declare no conflict of interest

FUNDING RESOURCES

The authors have no funding to report

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Citation:

Elsheikh, E., El bahrawy, A., Gawiesh, M., Doghda, A. The effect of biofilm on post-sinus surgical outcome in chronic rhinosinusitis. *Zagazig University Medical Journal*, 2022; (4127-4135): -. doi: 10.21608/zumj.2022.88292.2335