



Effect of Bacterial Biofilms on Disease Severity in Chronic Rhinosinusitis

Malik BA, Bhat MS, Altaf S, Khanam SF, Nisar J*, Khan MI, Farooq N and Qazi SM

Department of ENT and HNS, India

*Corresponding author: Jasif Nisar, Senior-Resident, department of ENT AND HNS, GMC Srinagar, India, Email: jasifnisar34@gmail.com

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Abstract

Introduction: Chronic rhinosinusitis (CRS) is an inflammatory disease affecting nose and para nasal sinuses for more than 12 weeks. Bacterial biofilms are emerging as an important etiological factor in CRS. The bacteria within the biofilm is resistant to treatment and adds to the severity of the disease in addition to posing difficulty in treating the disease.

Objective: The aim of the present study was to compare the Severity of disease in CRS Patients with and without bacterial biofilms.

Methods: Samples were taken from the patients and subjected to microbiological examination for biofilm detection. Lund Kennedy endoscopic scores and Lund Mackey CT scores were compared pre- treatment and at multiple follow ups.

Results: out of 60 patients 46 (32 (69.6%) patients were biofilm positive. Median MLK endoscopic scores for biofilm positive and biofilm negative patients were calculated and compared at pre-treatment, 1 month, 3 months and 6 months post treatment respectively $P < 0.001$. Lund Mackey CT score at pre-treatment and at 6 month post treatment follow up of biofilm positive and biofilm negative CRS patients was calculated. P value < 0.001 . Percentage improvement in Modified Lund Kennedy (MLK) endoscopic score was more for biofilm negative patients compared to biofilm positive patients.

Conclusion: Presence of biofilm on sinonasal mucosa is associated with more severe disease. Response to treatment is less in biofilm positive CRS patients. Presence of biofilms in CRS patients is an indication for more aggressive treatment.

Keywords: Lund Mackey; Medical and Surgical Treatments; Rhinosinusitis; Biofilm

Abbreviations: CRS: Chronic Rhinosinusitis; MLK: Modified Lund Kennedy; LK: Lund Kennedy; LM: Lund MacKay; CT: Computed Tomography; OPD: Outpatient Department; SDA: Sabouraud's Dextrose Agar; TCP: Tissue Culture Plating; TM: Tube Method; CRA: Congo Red Agar.

Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of nose and para nasal sinuses lasting for more than 12

weeks, a significant health problem affecting 5-12% of general population [1,2]. Underlying aetiology of chronic rhinosinusitis includes anatomical, functional (impaired mucociliary clearance), allergic, infective, environmental and more recently microbial biofilms [3,4]. The formation of bacterial biofilms on the sinus mucosa has been suggested to be responsible for chronic rhinosinusitis [5,6]. The first evidence of a potential role of biofilms in CRS came with the research of Ramadan, et al. [7]. Sanclement, et al. [5] concluded that biofilms may play a major role in the

pathophysiology of CRS in some patients. Bacteria within these biofilms are enclosed in polysaccharide matrix which renders them more resistant to host defences, such as immune system phagocytosis, and can be up to 1000 times more resistant to antibiotics [8-10]. Bacterial biofilms may be responsible for more severe disease in CRS patients compared to other etiologies. There are multiple scoring systems for assessing the severity of disease in CRS however two most widely used scoring systems are Lund Kennedy (LK) endoscopic score and Lund MacKay (LM) computed tomography (CT) score for endoscopic visualization of nasal cavity and CT findings respectively. The Lund Kennedy endoscopy scoring system grades visual pathologic states within the nose and paranasal sinuses including polyps, discharge, edema, scarring, and crusting [11]. Psaltis, et al. [12] described a modified Lund-Kennedy score which includes polyps, edema and discharge and has a high inter-rater and test-retest reliability. More, the modified Lund-Kennedy endoscopic score correlates well with the SNOT-22 score that is used to assess quality of life in CRS patients and also can be used to find the subjective severity of the disease. Endoscopic grading systems are very useful in the pre- and post-endoscopic sinus surgery research settings [11]. Computed tomography (CT) is considered the gold standard imaging modality for evaluation of sinonasal disease [13]. The Lund Mackey (LM) score is widely used and perhaps the best known method for radiological staging or quantifying the disease in chronic rhinosinusitis. Lund Mackey (LM) score measures a different aspect of chronic rhinosinusitis to subjective measures of disease severity [14]. The aim of the present study was to compare the severity of disease in chronic rhinosinusitis patients with and without bacterial biofilms using endoscopic and computed tomography scores.

Method

Sixty patients who reported to ENT & HNS outpatient department (OPD) of tertiary care hospital and diagnosed of CRS according to "clinical practice guidelines" 2015 criteria, and were willing to participate in the study.

- **Inclusion criteria:** i) Patients fulfilling the diagnostic criteria of CRS with or without nasal polyposis. ii) Age ≥ 18 to ≤ 60 .
- **Exclusion criteria:** i) Patients with fungal rhinosinusitis. ii) Patients with sinonasal malignancy. iii) Patients of CRS with medical comorbidities

Patients who were diagnosed with Chronic rhinosinusitis according to AAO HNS clinical practice guidelines update 2015

criteria, were subjected to complete examination including general physical examination, systemic examination and ENT examination. Nasal endoscopy was done to document inflammation and to record the modified Lund Kennedy endoscopic scores. Computed tomography was done to document inflammation and to record Lund Mackey CT score. Samples from patients of CRS were taken in the form of swab of nasal secretions and /or crusts from nose and/or tissue (mucosa or polyps) and transported immediately to microbiology laboratory.

Microbiological Examination

Samples were inoculated on 5% sheep blood agar, MacConkey and chocolate agar for bacterial culture and on sabouraud's dextrose agar (SDA) for fungal culture. Plates were incubated at 37^oc for 24 hours. Organisms were identified by standard microbiological procedures including various biochemical tests. All isolates were subjected to antimicrobial susceptibility testing on Mueller Hinton agar based on CSLI guidelines. Isolates were then tested for in vitro production of biofilm by following three methods. i) Tissue culture plating (TCP) method. ii) Tube method (TM). iii) Congo red agar (CRA) method.

Results

In this study, 60 patients were enrolled. Maximum number i.e. 19 patients (31.67%) were in the age group of 21-30 years. Minimum number i.e. 6 (10%) patients were in the age group of 51-60. Mean age in years was 33.05 \pm 12.216. Thirty seven (61.7%) patients were males and 23 (38.3%) patients were females with male: female ratio of 1.6:

All the study patients were subjected to microbiological examination for detection of bacterial biofilms (Table 1).

Biofilm	No of patients	Overall Percentage
Positive	32	53.3
Negative	28	46.7
Total	60	100

Table 1: Distribution of presence / absence of Biofilms among study participants (N=60).

Biofilms were present in 32 (53.3%) patients whereas in 28 (46.7%) patients biofilms were absent. Modified Lund Kennedy (MLK) endoscopic score of biofilm positive CRS patients was calculated pre-treatment and at multiple follow ups (1 month, 3 months, and 6 months) (Table 2).

Modified Lund Kennedy Score	N	Mean	SD	Min	Max	Median
Pre-treatment	32	7.59	2	4	12	7.5
At 1 Month follow up	32	3.53	1.27	1	7	3
At 3 Months follow up	32	4.22	1.96	0	9	4
At 6 Months follow up	32	4.09	2.05	0	8	4

Table 2: Comparison of Modified Lund Kennedy Endoscopic Score Pre-treatment with 1 month, 3 months & 6 months post-treatment among Biofilm positive subjects: (N = 32).

Mean, median, minimum and maximum scores were calculated at each visit. Mean (\pm SD) MLK score pre-treatment, at 1 month, 3 months and 6 months follow up post treatment were 7.59 (\pm 2.00), 3.53 (\pm 1.27), 4.22 (\pm 1.96) and 4.09 (\pm 2.05) respectively. Minimum score at pre-treatment was 4 and at 1 month was 1. At 3 months and 6 months the score was 0 for each. The Maximum score at pre-treatment and at 1 month, 3 months and 6 months follow up post treatment was 12, 7, 9 and 8 respectively. Median MLK score was 7.5, 3, 4

and 4 pre-treatment, 1 month, 3 months and 6 months follow up post treatment respectively. P Value < 0.001 was observed at all the three follow-ups using Wilcoxon Signed Ranks Test, which is statistically significant.

Modified Lund Kennedy (MLK) endoscopic score of biofilm negative CRS patients was calculated pre-treatment and at multiple follow ups (1 month, 3 months, and 6 months) (Table 3).

Modified Lund Kennedy Score	N	Mean	SD	Min	Max	Median
Pre-treatment	28	6	2.39	2	12	6
At 1 Month follow up	28	3.68	1.56	0	7	4
At 3 Months follow up	28	2.75	2.01	0	7	2.5
At 6 Months follow up	28	3.21	2.32	0	8	3

Table 3: Comparison of Modified Lund Kennedy Endoscopic Score Pre-treatment with 1 month, 3 months & 6 months post-treatment among Biofilm negative subjects (includes 14 culture positive and 14 culture negative patients). (N=28).

Mean, median, minimum and maximum scores were calculated at each visit. Mean (\pm SD) MLK score pre-treatment at 1 month, 3 months and 6 months follow up post treatment were 6.00 (\pm 2.39), 3.68 (\pm 1.56), 2.75 (\pm 2.01) and 3.21 (\pm 2.32) respectively. Minimum score at pre-treatment was 2, at 1 month 3 months and 6 months follow up the score was 0 for each. The Maximum score at pre-treatment and at 1 month, 3 months and 6 months follow up post treatment was 12, 7, 7 and 8 respectively. Median MLK score was 6.00, 4.00, 2.50

and 3.00 pre-treatment, 1 month, 3 months and 6 months follow up post treatment respectively. P Value < 0.001 was observed at all the three follow-ups using Wilcoxon Signed Ranks Test, which is statistically significant.

Lund Mackey CT score at pre-treatment and at 6 month post treatment follow up of biofilm positive CRS patients was calculated (Table 4).

Lund Mackey CT Score	N	Mean	SD	Min	Max	Median
Pre-treatment	32	13	4.69	3	24	14
6 Months Post-treatment.	32	7.56	4.28	1	19	6.5

CT: computed tomography, SD: standard deviation, Min: minimum, Max: maximum.

Table 4: Lund Mackey CT Score at Pre-treatment and 6 months follow up among Biofilm positive subjects (N=32).

In 32 Biofilm positive study subjects with mean \pm SD scores at pre-treatment and at 6 months were 13.00 \pm 4.69 and 7.56 \pm 4.28 respectively. Z value of -4.589 was observed. P Value < 0.001 was observed using Wilcoxon Signed Ranks Test which is statistically significant.

Lund Mackey CT score pre-treatment and at 6 month post treatment follow up of biofilm negative CRS patients was calculated (Table 5).

Lund Mackey CT Score	N	Mean	SD	Min	Max	Median
Pre-treatment	28	8.54	3.33	4	18	8
6 Months Post-treatment.	28	3.96	2.53	0	10	4

CT: computed tomography, SD: standard deviation

Table 5: Lund Mackey CT Score at Pre-treatment and 6 months follow up among Biofilm negative subjects (includes 14 culture positive and 14 culture negative patients). (N=28)

In 28 Biofilm negative study subjects with mean \pm SD scores pre-treatment and at 6 months were 8.54 ± 3.33 and 3.96 ± 2.53 respectively. Minimum score pre-treatment and 6 months follow up was 4 and 0 respectively. Maximum score pre-treatment and 6 months follow up was 18 and 10 respectively. Median score pre-treatment and 6 months follow up was 8.00 and 4.00 respectively. **Z value of -4.589**

was observed. P Value was $<.001$.

Improvement of Modified Lund Kennedy (MLK) endoscopic scores between biofilm positive 32 (53.3%) patients and biofilm negative 28 (46.7%) patients was compared (Table 6).

Biofilm	N	Mean	Standard Deviation	Mean Difference	p Value
Negative	28	-96.79	2.32	-0.879	0.124
Positive	32	-95.91	2.05		

Table 6: Comparison between percentage improvement in Modified Lund Kennedy (MLK) endoscopic Score at 6 months post-treatment among Biofilm positive & Biofilm negative CRS patients(N=60).

Mean percent improvement in biofilm positive patients was -96.79 ± 2.32 and in biofilm negative patients it was -95.91 ± 2.05 . The mean difference was -0.879 . Biofilm positive CRS patients show lesser improvement in MLK Score with p value as 0.124 which is not statistically significant.

Percentage improvement of Lund Mackey CT score between Biofilm positive 32 (53.3%) patients and biofilm negative 28 (46.7%) patients was compared (Table 7).

Biofilm	n	Mean % Improvement	Std. Deviation	Mean Difference	p Value
Negative	28	-96.036	2.53	-3.598	< 0.001
Positive	32	-92.438	4.28		

Table 7: Comparison between percentage improvement in Lund Mackey CT Score at 6 months post-operative among Biofilm positive and Biofilm negative CRS patients (N=60).

Mean percent improvement in Biofilm positive patients was $-(\text{minus}) 92.438 \pm 4.28$ and Biofilm negative patients was $-(\text{minus}) 96.036 \pm 2.53$. **A mean difference of -3.598 was observed with p value as < 0.001 which is statistically significant.**

in Chronic Rhinosinusitis without Nasal Polyposis" observed mean age of patients as 38.1 years (range, 24-53 years). Age distribution and mean age in this study was more or less similar to above reported studies.

Discussion

Maximum number i.e. 19 (31.67%) patients were in the age group of 21-30 years. Minimum number 6 (10%) patients were in the age groups of 51-60. Mean age in years was 33.05 with standard deviation of ± 12.216 . A study conducted by Bezerra, et al. [15] titled "Biofilm in Chronic Sinusitis with Nasal Polyposis" in which patients were in the range of 22-60 years with a mean age of patients as 37 years ± 11 years. Another study by Tatar EC, et al. [16] titled "Prevalence of Bacterial Biofilms and Their Response to Medical Treatment

Distribution of Patients on the basis of Presence or Absence of Biofilms (Table 1)

In our study of 60 patients, 32 (53.3%) patients were biofilm positive whereas 14 (47.7%) patients were biofilm negative. In a similar study by Sanderson AR, et al. [6] [14/39] titled "Bacterial Biofilms on the Sinus Mucosa of Human Subjects with Chronic Rhinosinusitis. Bacterial biofilms were present on 14 (77.77%) of the 18 CRS specimens. In another study by Tatar EC, et al. [16] titled, "Prevalence of Biofilms and Their Response to Medical Treatment in Chronic Rhinosinusitis without Polyps" determined that

biofilms were present in 75% of CRS patients. In another study done by P Singh, et al. [17] titled, "Bacterial biofilm on the sinus mucosa of healthy subjects and patients with chronic rhinosinusitis (with or without nasal polyposis)", 100 cases (50 control group and 50 disease group cases) were analyzed for the presence of biofilm. Bacterial biofilm was present in 26 (52%) out of 50 cases in the disease group.

Comparison of Modified Lund Kennedy Endoscopic Score at Pre-Treatment with 1 Month, 3 Months & 6 Months Post-Treatment among Biofilm Positive Subjects (Table 2)

In our study Modified Lund Kennedy (MLK) endoscopic score of Biofilm positive CRS patients (n=32) was calculated pre-treatment and at multiple follow ups (1 month, 3 months, and 6 months). Mean, median, minimum and maximum scores were calculated at each visit. Mean(\pm SD) SNOT-22 score pre-treatment, at 1 month, 3 months and 6 months were 7.59 (\pm 2.00), 3.53 (\pm 1.27), 4.22 (\pm 2.1.96) and 4.09 (\pm 2.05) respectively. Minimum score at pretreatment was 4 and at 1 month was 1. At 3 months and 6 months the score was 0 for each. The Maximum score at pre-treatment and at 1 month, 3 months and 6 months was 12, 7, 9 and 8 respectively. Median MLK score was 7.5, 3, 4 and 4 at pre-treatment, 1 month, 3 months and 6 months post treatment respectively. P Value <.001 was observed at all the three follow-ups using Wilcoxon Signed Ranks Test, which is statistically significant. An improvement in the scores indicated the response to treatment medical or surgical. In a study by done by Singhal D, et al. [18] titled "The impact of biofilms on outcomes after endoscopic sinus surgery" The biofilm-positive patients had median (with interquartile range) Kennedy-Lund scores of 4 (3-7) at 2 weeks, 4 (2-10) at 6 weeks, 3 (2-7) at 6 months. In our study the Modified Lund Kennedy (MLK) endoscopic score of biofilm positive CRS patients was more or less similar to above mentioned study.

Comparison of Modified Lund Kennedy Endoscopic Score Pre-Treatment with 1 Month, 3 Months & 6 Months Post-Treatment among Biofilm Negative Subjects (Table 3)

In this study Modified Lund Kennedy (MLK) endoscopic score of Biofilm negative CRS patients was calculated pre-treatment and at multiple follow ups (1 month, 3 months, and 6 months). Mean, median, minimum and maximum scores were calculated at each visit. Mean(\pm SD) MLK score pre-treatment, at 1 month, 3 months and 6 months follow up post treatment were 6.00 (\pm 2.39), 3.68 (\pm 1.56), 2.75 (\pm 2.01) and 3.21 (\pm 2.32) respectively. Minimum score at pre-treatment was 2, at 1 month 3 months and 6 months the score was 0 for each. The Maximum score at pre-treatment and at 1 month,

3 months and 6 months follow up post treatment was 12, 7, 7 and 8 respectively. Median MLK score was 6.00, 4.00, 2.50 and 3.00 pre-treatment, 1 month, 3 months and 6 months follow up post treatment respectively. P Value < 0.001 was observed at all the three follow-ups using Wilcoxon Signed Ranks Test, which is statistically significant. In a study by done by Singhal D, et al. [18] titled "The impact of biofilms on outcomes after endoscopic sinus surgery" The Biofilm-negative patients had median (with interquartile range) Kennedy-Lund scores of 2.5 (0.75-4) at 2 weeks, 0(0-2) at 6 weeks, 0 (0-3.8) at 6 months. The results in our study were different than other studies which may be due to more updated scoring systems used in our study.

Lund Mackay CT Score at Pre-treatment & 6 months Follow Up among Biofilm Positive Subjects (Table 4)

In this study Lund Mackey CT score at pre-treatment and at 6 month post treatment follow up of Biofilm positive CRS patients was calculated. In 32 biofilm positive study subjects with mean \pm SD scores at pre-treatment and at 6 months were 13.00 \pm 4.69 and 7.56 \pm 4.28 respectively P value < 0.001 was observed using Wilcoxon Signed Ranks Test which is statistically significant. Similar results were obtained by Jung JH, et al. [19] in their study "Clinical Characteristics of Biofilms in Patients with Chronic Rhinosinusitis: A prospective case-control study" the mean (\pm SD) LM CT score pre-treatment of biofilm positive patients was 18.07(\pm 3.24) and post treatment 6 month follow up mean (\pm SD) LM CT score was 12.12(\pm 4.81) with a p value of <0.001. In our study the results of LM CT score were more or less similar to the above mentioned study.

Lund Mackey CT Score Pre-treatment & 6 Months Follow Up among Biofilm Negative Subjects (Table 5)

In this study Lund Mackey CT score pre-treatment and at 6 month post treatment follow up of biofilm negative CRS patients was calculated. In 28 biofilm negative study subjects with mean \pm SD scores pre-treatment and at 6 months were 8.54 \pm 3.33 and 3.96 \pm 2.53 respectively. Minimum score pre-treatment and 6 months follow up was 4 and 0 respectively. Maximum score pre-treatment and 6 months follow up was 18 and 10 respectively. Median score pre-treatment and 6 months follow up was 8.00 and 4.00 respectively. Z value of -4.534 was observed. P Value < 0.001 was observed using Wilcoxon Signed Ranks Test which is statistically significant. In a study by Jung JH, et al. [19] titled "Clinical Characteristics of Biofilms in Patients with Chronic Rhinosinusitis: A Prospective Case-Control Study" the mean (\pm SD) LM CT score pre-treatment of biofilm negative patients was 10.18

(± 2.48) and post treatment 6 month follow up mean (\pm SD) LM CT score was $7.57(\pm 2.35)$ with a p value of < 0.001 .

Comparison between Percentage Improvement in Modified Lund Kennedy (MLK) Endoscopic Score at 6 Months Post- Treatment among Biofilm Positive & Biofilm Negative CRS Patients (Table 6)

In this study percentage improvement of Modified Lund Kennedy (MLK) endoscopic scores between biofilm positive 32 (53.3%) patients and Biofilm negative 28 (46.7%) patients was compared. Mean percent improvement in biofilm positive patients was -96.79 ± 2.32 and in Biofilm negative patients it was -95.91 ± 2.05 . The mean difference was -0.8790 . Biofilm positive CRS patients show lesser improvement in MLK Score with p value as 0.124 which is not statistically significant. In a study by done by Singhal D, et al. [18] titled "The impact of biofilms on outcomes after endoscopic sinus surgery" found a statistically significant difference between the improvement in nasal endoscopy scores of biofilm positive and biofilm negative patients. In another study by Jung JH, et al. [19] in their study "Clinical Characteristics of Biofilms in Patients with Chronic Rhinosinusitis: A Prospective Case- Control Study" found that the sinus Lund-Kennedy endoscopic score was significantly higher in the biofilm-positive group than the biofilm-negative group.

Comparison between Percentage Improvement in Lund Mackey CT Score at 6 Months Postoperative among Biofilm Positive & Biofilm Negative CRS Patients (Table 7)

In this study percentage improvement of Lund Mackey CT score between Biofilm positive 32 (53.3%) patients and Biofilm negative 28 (46.7%) patients was compared. Mean percent improvement in Biofilm positive patients was $-(\text{minus}) 92.438 \pm 4.28$ and Biofilm negative patients was $-(\text{minus}) 96.036 \pm 2.53$. A mean difference of -3.598 was observed with p value as < 0.001 which is statistically significant. In a study by Jung JH, et al. [19] in their study "Clinical Characteristics of Biofilms in Patients with Chronic Rhinosinusitis: A Prospective Case-Control Study" the mean (\pm SD) LM CT score pre-treatment of biofilm positive patients was $18.07 (\pm 3.24)$ and for biofilm negative patients the mean (\pm SD) LM CT score was $10.18 (\pm 2.48)$ with a p value of < 0.001 . In another study done by Psaltis AJ, et al. [20] titled "The effect of bacterial biofilms on post-sinus surgical outcomes" the mean CT scores of biofilm positive and biofilm negative patients were 18.5 and 14.5 respectively. The CT scores of biofilm positive patients were higher compared to biofilm negative CRS patients. In our study there was statistically significant difference in CT score improvement between

biofilm positive and biofilm negative CRS patients which was consistent with above mentioned studies.

Conclusion

Biofilms are an important factor in the etiology of CRS. Presence of biofilm on sinonasal mucosa is associated with more severe disease before treatment. Biofilm positive patient's shows less improvement in disease following treatment whether medical or surgical compared to their biofilm negative counterparts. Improvement in severity of disease was less in Biofilm positive CRS patients as indicated by Modified Lund Kennedy (MLK) Endoscopic scores and Lund Mackey (LM) CT scores. Presence of biofilms in CRS patients is an indication for more aggressive treatment compared to biofilm negative counterparts.

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