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**Original Research Article**

# Effect of medical treatment on eradication of biofilms in chronic rhinosinusitis

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## ABSTRACT

**Background:** Long-term macrolides and intranasal corticosteroids have evidence-based recommendations. It has been reported that macrolide therapy caused objective and subjective improvements which correlated to the duration of therapy. Topical nasal steroids are the mainstay of treatment in CRS. It is proposed that intranasal steroid may increase the restoration of normal epithelium.
**Methods**: Sample size of 24 in each group is sufficient to detect the difference between positivity rate with 80% power and 5% level of significance clinically diagnosed cases of CRS with nasal polyps in age group of 18-60 years were taken. Outcome was measured with multiple scoring systems like SNOT-20, CT scoring, endoscopic scoring system.
**Results**: Significant improvement was seen in the SNOT-20 scores with p value of 0.011 and endoscopic scores with p value of 0.001 in group having macrolides along with steroids pre-operatively however the scores post-surgery didn't show any significant change in both groups. The efficacy of macrolide with nasal steroidal spray or nasal spray alone showed no further benefit in the subjective outcome measures post operatively. Also, no statistically significant eradication of biofilms or decrease in density could be appreciated in both groups. Furthermore, there was no significant difference in recurrence rate.
**Conclusions**: Our result demonstrated the subjective improvement in patients of macrolide group post treatment which could be attributed to its anti-inflammatory effect. Even though macrolides in combination with nasal spray reflected some improvement in the secondary outcomes but the primary outcome of eradication of biofilms couldn't be achieved.

**Keywords:** Macrolide, Nasal steroids, Nasal polyp, Biofilm, Chronic rhinosinusitis

## INTRODUCTION

In the current literature, anti-inflammatory treatments such as long-term macrolides and intranasal corticosteroids have evidence-based recommendations from consensus and task force groups. Macrolides work primarily through their antibacterial effects as well as their anti-inflammatory effect It has also been reported that macrolide therapy caused objective and subjective improvements and was correlated to the duration of therapy and inhibiting cytokine, alter biofilm formation, increase cell apoptosis, inhibit activation of pro-inflammatory transcription factor nuclear factor-kB. Topical nasal steroids are the mainstay of the anti-inflammatory treatment in CRS. Sub epithelial elements which may act as receptors for the bacteria, can be exposed by the breaks in the epithelium. It is proposed that intranasal steroid usage may increase the restoration of normal epithelium by reducing mucosal inflammation.[1] The role of biofilms in otolaryngologic infections was first described by Cryer et al wherein biofilm was considered as playing a role in the recalcitrant nature of disease.[2] The

goal of this study is to compare the efficacy of topical nasal steroids either as monotherapy or combined with macrolides for biofilm eradication in patients of chronic rhinosinusitis with nasal polyps. A variety of subjective and objective measures have been used.

## METHODS

### *Subjects*

This randomised control study was conducted in the department of otorhinolaryngology and department of microbiology at university college of medical sciences and Guru Teg Bahadur Hospital, Delhi over an 18-month period (November 2015 to April 2017). Patients included were of age group 18-60 years. They were subjected to history and using visual analogue scale for SNOT-20 questionnaire, routine ENT examination including anterior rhinoscopy and posterior rhinoscopy, blood investigations including absolute eosinophil count, computed tomography of paranasal sinuses- axial, coronal and sagittal views (Lund & Mackey CT scoring) and diagnostic nasal endoscopy and tissue sample for biofilm presence.[3,4] Chronic rhinosinusitis (CRS) cases with bronchial asthma, immunocompromised patients and pregnant females, mucociliary function dysfunction (e.g. cystic fibrosis, Kartagener syndrome) and secondary causes of chronic rhinosinusitis were excluded. Patients taking topical nasal spray and antibiotics for last one month were excluded from this study. The study protocol was approved by the ethics committee of Delhi University.

### *Experimental procedures*

After recruitment into the study, subjects were randomized into two groups, one receiving tab Clarithromycin 250mg OD along with mometasonefuroate nasal spray (MFNS) single dose of 200mcg/day and the second receiving mometasonefuroate nasal spray single dose of 200mcg/day alone for 4 weeks. investigators and patients were kept blinded to the randomization till the end of the study. At the beginning and end of treatment, patients completed the sinonasal outcome test-20, general ear, nose, throat examination and diagnostic nasal endoscopy scoring by Lund and Kennedy.[5] Tissue sample for biofilm detection will be taken under topical and infiltrative anaesthesia and sent to Microbiology department both before initiation of medical management and surgery to access the end point biofilm presence. In addition, SNOT-20 questionnaire along with diagnostic endoscopy (DNE) was done 2 weeks, 1 month and 3 months after surgery.

### *Microbiological, subjective testing and measurements*

Tissue samples for biofilm detection was taken before and after treatment. sinonasal outcome test-20: patients completed the SNOT-20 question are at their enrolment into the study, after treatment and 2weeks, one month and 3 months after the surgery. Visual analogue scale: symptoms were graded on visual analogue scale 0 to 5 for severity.

### *DNE*

Patients' nasal cavity was examined with nasal endoscope and was graded according to Lund and Kennedy scoring at pre and post treatment and also after 2 weeks, 1 month and 3 months after surgery. Endoscopic scoring was carried out according to template grading nasal polyp (0-absence of polyps, 1-polyps within the middle meatus, 2-polyps beyond middle meatus), oedema, scarring and crusting (0-absent, 1-mild, 2-severe), discharge (0-absent, 1-present, 2-thick purulent discharge).

### *Statistical methods*

Sample size of 24 in each group was found to be sufficient to detect the difference between positivity rate with 80% power and 5% level of significance. So, sample size of 24 in each group was taken. Chi-square/Fisher exact test to compare the biofilm positivity rate between the two groups. Unpaired student T test to compare the variables like biochemical parameters and Chi-Square test/Fisher test for qualitative variables like clinical, radiological and endoscopic features in chronic rhinosinusitis (CRS) patients with biofilm positivity or biofilm negativity. Data are expressed as mean±standard of mean, p values <0.05 was considered to be significant.

## RESULTS

### *Subjects*

Total 48 patients of CRSwNP were selected as study group. Of these 48 patients 24 each were further randomly divided into two groups, one which underwent medical management with mometasonefuroate nasal spray along with low dose macrolide clarithromycin (Group 1), other with mometasonefuroate nasal spray alone (Group 2).

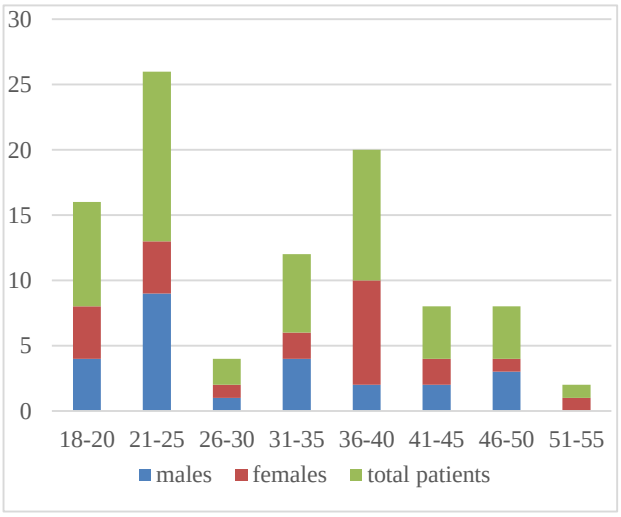


**Figure 1: Age and sex distribution in CRSwNP.**

**Table 1: Pre versus post treatment outcomes in both groups.**

| Groups | Pre- treatment | Post- treatment | First visit | Post- op Second visit | Third visit |
|---|---|---|---|---|---|
| **Group 1** | 28.05±6.772 | 17.91±5.648 | 3.55±2.365 | 5.82±3.418 | 9.45±4.273 |
| **Group 2** | 49.42±3.063 | 39.88±3.167 | 4.446±0.833 | 7.21±2.604 | 11.87±3.026 |
| **Critical difference** | 12.37 | 21.97 | 0.886 | 1.39 | 2.42 |

**Table 2: Comparison of endoscopic scores.**

| Groups | Pre- treatment | Post- treatment | First visit | Post- op Second visit | Third visit |
|---|---|---|---|---|---|
| **Group 1** | 7.83±1.173 | 4.92±0.830 | 1.00±0.00 | 1.17±0.381 | 1.38±0.495 |
| **Group 2** | 7.25±1.294 | 6.83±1.465 | 1.00±0.00 | 1.08±0.282 | 1.21±0.415 |
| **Critical difference** | 0.13 | 1.91 | 0 | 0.07 | 0.17 |

**Table 3: Comparison presence of biofilm in CRS patients and control of our study with previous studies.**

| Study | Biofilm in group 1 | Biofilm in group 2 | P value |
|---|---|---|---|
| **Our study** | 14/14 | 3/3 | 1.000 |
| **Korkmaz** | 6/12 | 1/11 | 0.005 |

**Table 4: Comparison of age group of our study with previous studies.**

| Study | Median age of group 1 | Median age of group 2 |
|---|---|---|
| **In our study** | 36 | 23 |
| **Haxel** | 45 | 47 |
| **Tartar** | 38 | 36 |

**Table 5: Comparison of sex ratio of our study with previous studies.**

| Study | Male: Female in group1 | Male:Female in group 2 |
|---|---|---|
| **In our study** | 0.8:1 | 1.4:1 |
| **Tartar** | 1.6:1 | 1.2:1 |
| **Haxel** | 1.4:1 | 1.4:1 |

These cases were evaluated preoperatively by clinical examination, SNOT-20, CT, endoscopic score and their polypoidal samples were sent for bacterial culture and biofilm detection.

Another sample was sent preoperatively following the medical management. These patients were further followed up in postoperative period and evaluated using SNOT-20 and endoscopic score. No significant differences were seen between the groups regarding age and sex. Bacterial culture was positive in 40 out of 48 samples. Most of the cultures i.e., 25 out of 40 revealed growth of bacteria Staphylococcus aureus out of which 2 were methicillin resistant. Pseudomonas species was found in 5 samples. Klebsiella species, and Coagulase positive Staphylococcus were found in 5 and 3 culture specimen each while E Coli was found in a single sample. There were 8 samples that did not have any growth. Hence a total of 17 patients out of 40 cases i.e., 42.5% were found to have biofilms. There was decrease in density of biofilm in a single patient in Group1 rest all there was no difference in density of biofilms in both groups. No macrolide resistant organ ISMS were noted to develop. SNOT-20 scoring: Nasal obstruction and need to blow nose were present in all the 48 patients i.e., 100% cases. The mean SNOT-20 score improved in group 1 from 28.05 to 17.91 (53%) but in group 2 improvement was less significant (40.42 to 39.88).

***Endoscopic scoring***

Significant difference could be appreciated between both the groups post treatment. According to Tukey's test, statistically significant difference is seen between pre-treatment and post treatment SNOT -20 scores in Group 1 while not much difference could be seen in Group 2. However, the post operative scores of both the groups were statistically similar. Statistically significant difference could also be appreciated in the endoscopic scores of Group1 and 2 post treatment. While the post operative picture was similar to SNOT-20 score i.e., not significant. After 3 months, 14 patients presented with recurrence or persistence of symptoms. These patients were evaluated again. They had individual SNOT-20 score of more than 8

and increased Lund-Kennedy score in diagnostic nasal endoscopy. Recurrence was found in 7 out of 17 patients (41.17%) who were positive for biofilm in Group 1 and however recurrence was seen in 5 patients in Group 2 even though they were negative for biofilms. These patients were started on oral steroids and antibiotics based on sensitivity results. However, no significant correlation between recurrence rate and presence of biofilm was seen, the p value was 0.033.

## DISCUSSION

Role of biofilms in CRS is being increasingly recognised in the medical literature. Since biofilms were initially reported in patients who had sinus surgery and were resistant to medical therapy, they have been studied in various clinical trials of CRS. In our study the prevalence of biofilms in CRS has been reported in nearly 35% of out-patient population depending on many factors such as number of samples, inclusion criteria of patients, imaging modalities and examination. Now a days bacterial biofilms have been implicated in the chronic nature of CRS. Studies have shown that patients with biofilm have more persistent postoperative symptoms, ongoing inflammation, and infections. Zhang et al reported that biofilms were found in nasal and sinus mucosa of CRS patients before and after FESS and they contribute to an unfavourable outcome after surgery.[6] However, in our study we characterized the presence of biofilms in the nasal polypoidal tissues of 48 patients with chronic rhinosinusitis and found that 17 out of 48(35.41%) samples were positive for biofilm of which 14 were in group 1 and 3 were group 2, but no regress was seen in biofilm presence in both groups following treatment. Hence no significant improvement was seen(p=1.000). However, in previous study by Korkmaz et al, eradication of biofilm was evident in 6 of 12 (50%) in group receiving clarithromycin along with mometasone while improvement was seen in 1 of 11 patients in group on mometasone nasal group.[7]

Most common organism that forms biofilm in both the groups were Staphylococcus aureus. 41% of samples of clarithromycin group and 21% of samples of nasal spray group were biofilm producing Staphylococcus aureus. Those Staphylococcus aureus with biofilm forming capacity found in patients may in future lead to CRS. So, these control patients need long term follow up and further study. In a study conducted by Sachse F et al they found that Staphylococcus aureus was the frequent bacterial isolates in CRS and was frequently seen in polymicrobial biofilms[8]. It is also believed to play a role in the pathogenesis of CRS by producing toxins and inducing specific immunity to superantigen. We included 48 patients of which 24 patients were allocated to the group 1 with median age of 36 years while 24 patients were allocated to group 2 with median age group of 23 years. Out of the 24 patients in group 1, 13 were females and 11 were males while in group 2, 10 were females while 14 were males. Comparison of age group and sex ratio of CRS patients of our study with other studies Haxel et al and Tartar et al are shown.[9,10]

**Table 6: Comparison of preoperative mean SNOT-20 of our study with other studies.**

| Study | Group 1 | Group 2 | P value |
|---|---|---|---|
| **Our study** | 28.05 to 17.91 | 40.42 to 39.88 | <0.011 |
| **Korkmaz** | 26.5 to17.6 | 32.6to 21.2 | 0.001 |
| **Haxel** | 21.5 to 13.7 | 19.7 to 9.3 | 0.555 |

**Table 7: Comparison of postoperative mean change of SNOT-20 from visit1 and visit2 of our study with other studies.**

| Study | Group 1 | Group 2 | P value |
|---|---|---|---|
| **Our study** | 3.55±2.36 | 4.46±0.833 | 0.011 |
| **Haxel** | 3.7±10.4 | 5.3±12.6 | 0.6230 |

**Table 8: Comparison of mean of endoscopic score between our study and other studies.**

| Study | Group 1 | Group 2 | P value |
|---|---|---|---|
| **Our study** | 1.00±0. 00 | 1.00±0.00 | 0.033 |
| **Haxel** | 2.6±1.4 | 2.5±1.3 | <0.05 |

**Table 9: Comparison of mean change of endoscopic score of visit1 and visit2 between our study and other studies.**

| Study | Group 1 | Group 2 | P value |
|---|---|---|---|
| **Our study** | 0.17±0.381 | 0.08±0.282 | 0.033 |
| **Haxel** | 0.7±0.2 | 0.1±0.2 | 0.035 |

**Table 10: Comparison of mean CT scores of group1 with group 2 between our study and other studies.**

| Study | Group 1 | P value | Group 2 | P value |
|---|---|---|---|---|
| **Our study** | 11.66±0.766 | 0.062 | 6.36±3.86 | 0.215 |
| **Korkmaz** | 2.58±0.61 | 0.02 | 2.60±0.51 | 0.007 |

The most common symptom with which patient presented was nasal obstruction and the need to blow nose which was present in 100% patients. The other common symptoms were thick nasal discharge, post nasal discharge and running nose. CRS also has impact on social and emotional life of patients as can be seen from the observation that patients had reduced concentration and reduced productivity in their routine work. Also, most of them were sad, frustrated and embarrassed due to this disease. On evaluation of SNOT -20 score a significant difference was seen in patients on clarithromycin, mean decreased from 28.05 to 17.91 showing a statistically significant difference (p<0.011) also in nasal spray group difference from 40.42 to 39.88 was seen. Similar difference was also seen in study conducted by Korkmaz et al in which mean

SNOT-20 sore was improved from 26.5 to 17.6 (p value 0.001). Improvement was also seen in another study conducted by Haxel et al in which improvement in SNOT 20 was seen from 21.5±13.7 to 19.7±9.3 (p value 0.555) in similar groups.

Our post operative endoscopic score of groups 1 showed significant improvement in comparison to group 2 as is in conjunction with study done by Haxel et al. Our study showed significant difference in the SNOT-20 scores of between pre and post clarithromycin group which is in conjunction with other studies like Haxel and Korkmaz. Post operatively this difference was however not much appreciated. Similarly in endoscopic score the group 1 showed statistically significant difference which was also seen in other studies. However, the post operative endoscopic finding in first and second visit were more or less similar showing no significance. In addition, we found that there was no significant difference in recurrence of disease between the groups (p=0.398). Our study found that the prevalence of biofilm in patients of group 1 was significantly different from that of group 2. In case of recurrent episodes of sinusitis, sinuses have tendency to become colonized by various types of microbes. Therefore, it is now thought that the etiopathological factors playing a role in the development of CRS could be a bacterial biofilm. Biofilm as a single agent is not responsible for the manifestation of CRS. But they depend on other cofactors. Host factors could also be responsible for pathogenesis of biofilm.

## CONCLUSION

Our study group comprising of 48 patients showed significant improvement in the SNOT-20 scores and endoscopic scores in group having macrolides along with steroids pre-operatively however the scores post-surgery didn't show any such significant change in both groups. The efficacy of macrolide with nasal steroidal spray or nasal spray alone showed no further benefit in the subjective outcome measures post operatively. Also, no statistically significant eradication of biofilms or decrease in density could be appreciated in both groups. Furthermore, there was no significant difference in recurrence rate. Even though macrolides in combination with nasal spray reflected some improvement in the secondary outcomes but the primary outcome of eradication of biofilms couldn't be achieved. Hence more research is needed so as to answer this topic and confirm the effectiveness of clarithromycin on mucosal biofilms.

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