Research

Effects of topical hyaluronic acid on nasal mucosa wound healing

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ABSTRACT

Background: Chronic rhinosinusitis (CRS) is a chronic inflammatory disease of the nasal and paranasal sinuses mucosa that takes place for more than 3 months. The treatment paradigm nowadays depends on Functional Endoscopic Sinus Surgery (FESS) in the hope to repair the damaged mucosa, restoring it to its physiological condition. Hyaluronic Acid (HA) could restore the natural barrier of the mucosa and stop the inflammatory cascade. **Purpose:** To understand the difference in the number of inflammatory cells in the post-FESS nasal mucosa in CRS patients that had been treated with HA. **Method:** This was pre and post-test, double-blinded, randomized control group study design of 22 CRS patients (range 18 to 55 years) in Dr. Kariadi General Hospital, Semarang from May to August 2019. All subjects had undergone biopsies of the inferior turbinate at the time of surgery and 4 weeks after surgery. The number of pre and post-test neutrophil, eosinophil and lymphocyte counts were compared between control and treatment groups. The neutrophil-to-lymphocyte ratio (NLR) and eosinophil-to-lymphocyte ratio (ELR) levels were also compared between the two groups. Data analysis was done using the Wilcoxon test. **Result:** When the pre and post-test treatment group and the control group were compared, no statistically significant difference was found between two groups except for ELR in the treatment group (p=0.028). **Conclusion:** ELR was decreased significantly in the treatment group. NLR level was also decreased but not significant. This study had shown that topical HA could positively modulate the inflammatory response.

Keywords: chronic rhinosinusitis, FESS, histopathology, neutrophil, eosinophil, lymphocyte

ABSTRAK

Latar belakang: *Rinosinusitis kronis (RSK) merupakan penyakit inflamasi kronik pada mukosa hidung dan sinus paranasal yang berlangsung lebih dari 12 minggu. Bedah Sinus Endoskopi Fungsional (BSEF) pada RSK bertujuan untuk memperbaiki mukosa yang rusak dan mengembalikannya ke kondisi fisiologis. Asam hyaluronat (AH) dapat mengembalikan penghalang alami mukosa dan menghentikan proses inflamasi.* ***Tujuan:*** *Untuk mengetahui perbedaan jumlah sel inflamasi pada mukosa hidung pasca BSEF pada pasien RSK yang diberikan terapi tambahan AH.* ***Metode:*** *Penelitian ini merupakan penelitian pre dan post-test, tersamar ganda, desain penelitian kelompok kontrol acak terhadap 22 pasien RSK (berusia 18 hingga 55 tahun) di RS Dr. Kariadi, Semarang dari bulan Mei hingga Agustus 2019. Semua subjek dilakukan biopsi konka inferior pada saat operasi dan 4 minggu setelah operasi. Jumlah neutrofil, eosinofil, dan limfosit sebelum dan sesudah terapi dibandingkan antara kelompok kontrol dan kelompok perlakuan. Rasio neutrofil terhadap limfosit (NLR) dan rasio eosinofil terhadap limfosit (ELR) juga dibandingkan antara kedua kelompok. Analisis data dilakukan dengan menggunakan uji Wilcoxon.* ***Hasil:*** *Hasil perbandingan kelompok perlakuan dan kelompok kontrol sebelum dan sesudah terapi, menunjukkan tidak ada perbedaan yang signifikan secara statistik antara kedua kelompok kecuali ELR pada kelompok perlakuan (p=0,028).* ***Kesimpulan:*** *ELR menurun secara signifikan pada kelompok perlakuan. Tingkat NLR juga menurun tetapi tidak signifikan. Penelitian ini menunjukkan bahwa AH topikal dapat secara positif memodulasi respon inflamasi.*

Kata kunci: *rinosinusitis kronis, BSEF, histopatologi, neutrofil, eosinofil, limfosit*

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INTRODUCTION

Chronic rhinosinusitis (CRS) is a chronic inflammatory disease of the paranasal sinuses and nasal mucosa, that takes place for 3 months or more.[1] The treatment paradigm nowadays depends on Functional Endoscopic Sinus Surgery (FESS) and intranasal topical steroid in the hope to repair the damaged mucosa, restoring it to its physiological condition.[2,3] Although FESS was not aggressive, nasal mucosa would still be damaged, which must be followed by post-surgical wound healing. Wound healing is a highly organized process, involving inflammation, cell proliferation, matrix deposition or remodelling, and is regulated by a wide variety of growth factors. It generally occurs up to 6 months.[4] Dysregulation of any of these processes can result in delayed wound healing, and the potential to form complications post-FESS including synechiae and crust.

Hyaluronic acid (HA) is the main component of many extracellular matrices in the epithelial cells of the respiratory tracts, and serous glands of the nasal and trachea-bronchial mucosa. HA holds a significant biological role aside from its function as a structural component of the interstitial and connective tissue. There had been several studies existed about the effect of HA on respiratory tract infection, asthma, and allergic rhinitis. The application of topical HA could significantly increase the wound-healing process of the nasal mucosa, furthermore, it could inhibit the formation of excessive crusts, promote reepithelization after surgery, and reduce the risk of synechiae, edema, and mild mucopurulent secrets production.[5,6]

Study of HA effectivity in NaCl 0.9% used for nasal irrigation by nebulization in patients post-FESS had been done,[5,6]leading to bronchial hyperresponsiveness (BHR), but studies on the effect of topical HA as nasal drops on wound-healing process post-FESS had never been carried on.

The purpose of this study was to understand the difference in the number of inflammatory cells in post-FESS nasal mucosal histopathology, in patients with CRS that had been treated with HA.

METHOD

This was an interventional study with pre and post-test, double-blinded, randomized control group design. This study was approved by the Health Research Ethics Committee of Dr. Kariadi General Hospital, Semarang No.174/EC/KEPK-RSDK/2019. It was performed at ENT clinic, Central Operating Theatre, and Anatomic Pathology Laboratory of dr. Kariadi General Hospital, Semarang, in the period of May to August 2019.

A total of 22 subjects were included in this study. The sampling was done by consecutive sampling, in which all patients who came in a row, and fulfilled the inclusion criteria. Those were patients diagnosed CRS based on EPOS 2012, aged from 18-55 years old, and were approved for this study. The exclusion criteria were patients with sinonasal malignancy. Confounding variables were included: nasal polyp, allergy, diabetes mellitus, hypertension, tuberculosis, and smoking.

Subjects had given their written informed consent priorly, and then they underwent FESS. After surgery, the subjects received bottles of medications that had been numbered according to the randomization table with double blinding-randomized. The bottle containing HA 0.1% was given to the treatment group, and the bottle containing NaCl 0.9% was for the control group. All bottles had a similar shape, size, and color. The treatment group received standard therapy [cefadroxyl for 5-7 days, kalnex (antihemorrhagic), paracetamol, methylprednisolone (2 days), and nasal irrigation], added with HA 0.1%. While the control group received standard therapy only.

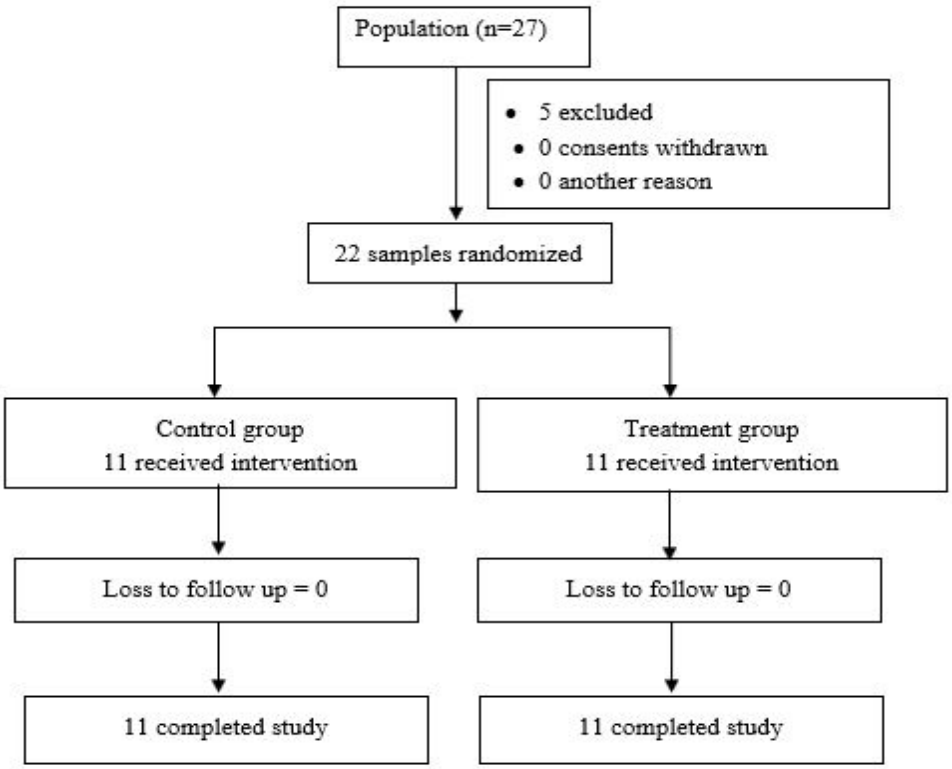


Figure 1. CONSORT diagram

Histopathology

Nasal mucosal biopsy was performed on each subject. The tissue was taken from the anterior part of the nasal inferior turbinate. The first biopsy was performed during FESS, and the second biopsy was done 4 weeks after FESS. The biopsy specimens were fixated in 10% *Buffered-Neutral Formalin* at room temperature. After fixation, tissue biopsies were processed using tissue processor (Excelsior^{Tm} AS tissue-processor, Thermo Fisher Scientific, WA, UK). The Formalin-Fixed Paraffin-Embedded (FFPE) tissues were cut by microtome with 4 μm thickness, and stained with Haematoxylin and Eosin (H&E).

The sample slides were examined by two pathologists in a blinded manner. The sample analysis was performed in 5 High power fields (400x magnification) to obtain the inflammatory cells (lymphocyte, eosinophil, and neutrophil), and the field was chosen with a maximal density of inflammatory cells. The Neutrophil-to-Lymphocyte Ratio (NLR) was determined simply by dividing the number of neutrophils by the number of lymphocytes, and

also Eosinophil-to-Lymphocyte Ratio (ELR) by dividing the number of eosinophils by the number of lymphocytes.

Collected data was checked for its completeness and validity, and then edited, coded, tabulated, and digitally recorded. The IBM SPSS (Statistical Product and Service Solutions) 15 version (Statistical Software, Chicago, USA) program was employed for evaluating the data gathered in this study. Comparative test analysis was performed using Wilcoxon with a significance level of <0.05.

RESULT

A total of 22 CRS patients consisted of 9 males and 13 females, with a mean age of 30.95 ±12.66 years. The control group was 11 subjects without HA, and the treatment group was 11 subjects who received HA 0.1% nasal drops. Subjects who suffered from allergic rhinitis were 63.6%, smoking 13.6%, and had nasal polyp 9.1%. The side effects occurring in this study were dry nose (13.6%). There were no epistaxis nor allergy side effects (Table 1).

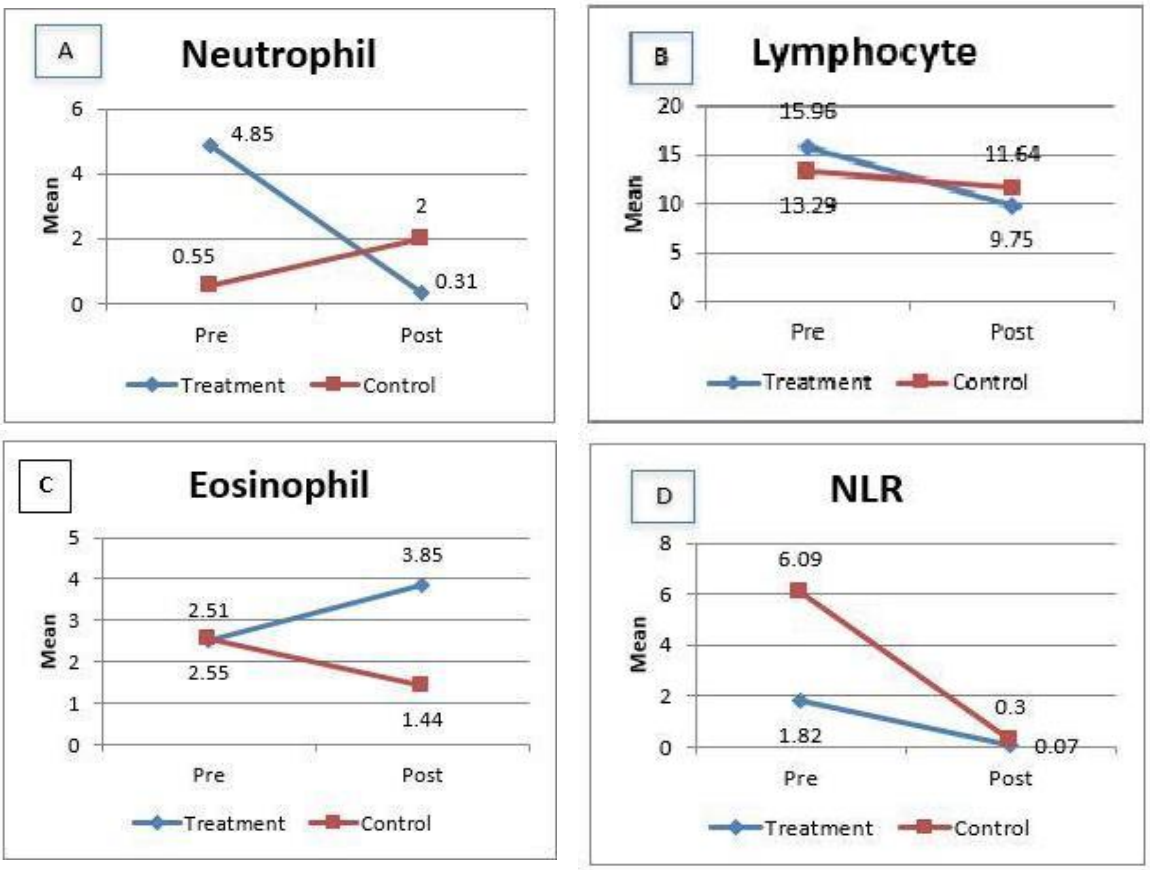
Table 1. Clinicopathological features of CRS patients (n = 22)

Variable	F	%	Mean ± SD	Median (min – max)
Sex				
Male	9	40 .9		
Female	13	59 .1		
Age Polyp			30.95±12.66	27(18 – 55)
No	20	90.9		
Yes	2	9.1		
DM				
No	22	100		
Yes	0	0		
Smoking				
No	19	86.4		
Yes	3	13.6		
Side effect				
No	19	86.4		
Yes	3	13.6		
Allergy				
No	8	36.4		
Yes	14	63.6		

Figure 2 showed neutrophil, eosinophil, and lymphocyte counts on nasal mucosal histopathology, and the levels of NLR and ELR. It showed a decreasing number of neutrophils in the treatment group before and after treatment, but statistically not significant (Wilcoxon test, p=0.893). There was an increase in the number of neutrophils in the control group before and after the treatment which was not significant (Wilcoxon test, p=0.093). There were differences in the number of neutrophils in the control group and the treatment group after treatment which was not significant, with p value >0.05.

While the number of eosinophils in the postoperative nasal mucosa in the treatment group and the control group in this study showed the opposite results from neutrophil counts. There was an increase in the number of eosinophils in the treatment group (deviation (Δ)= 1.35±9.15) and a decrease in the number of eosinophils in the control group after treatment compared to before treatment (deviation (Δ)= -1.11±2.43).

In accordance with the description of neutrophil counts, the number of lymphocytes in the control and treatment groups before and after treatment decreased (deviation (Δ)=-1.65±10.30 and -6.22±20.55) but not statistically significant (Wilcoxon test, p=1.000 and p=0.722). Furthermore, when the level of NLR and ELR pre and post-test treatment group and the control group were compared, no statistically significant difference was found between those two groups, except for ELR in the treatment group (p=0.028). The obtained data were summarized in Figure 2.



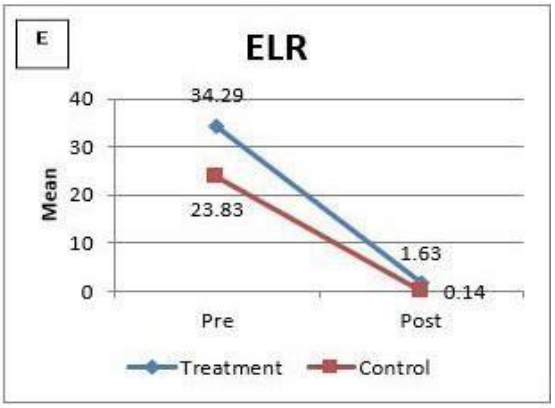


Figure 2. A. Mean Neutrophil, B. Mean Lymphocyte, C. Mean Eosinophil, D. Mean Neutrophil-to-Lymphocyte Ratio (NLR), E. Mean Eosinophil-to-Lymphocyte Ratio (ELR). ELR calculated from inferior turbinate mucosa was decreased significantly, while others were not significant.

DISCUSSION

Paranasal sinuses and nasal mucosa in CRS patient undergo the inflammatory process and remodelling.[1] The reversibility of the histopathological changes in CRS could inflict a significant clinical impact. FESS has been the standard of care in the treatment of CRS refractory to medical treatment. The surgery techniques, the presence of extensive crusts, impairment of ventilation of the paranasal sinuses, edema, and post-surgical bleeding, are problems that may present following FESS.[7]

Post-operative FESS care varied, included nasal saline irrigation, topical nasal steroids, antibiotics and mucolytic agents, as immediate postoperative care interventions.[8] In relation to this important issue, the use of HA had shown to be effective in increasing the wound-healing process of the nasal mucosa after FESS.[5,6] Side effects due to HA were included dry nose, epistaxis, and allergy. The effects occurring in this study was a dry nose.

Among the subjects of this study, there were more females than males both in the control group and treatment group. Several studies also yielded the same results as the one obtained in the study that took place in North Sumatera (2011), which reported that females were more prone to CRS (54.3%).[9] In Dr. Kariadi General Hospital Semarang in 2014, females were more likely to suffer from CRS (73.3%).[10]

The average age of CRS in previous references was 39.8 years old.[11] A study in North Sumatra (2011) reported that the onset of CRS was mostly at the age of 31 to 45 years old (31.6%).[9] In Dr. Kariadi General Hospital Semarang in 2014, CRS took place at the age of <45 years old (70%).[10] The average age of the subjects in this study was 34 years old for the control group, and 23 years old for the treatment group. The subjects of this study had risk factors of allergic rhinitis, smoking, and polyp. But no significant difference between treatment and control groups, so that the risk factors in this study did not influence the outcomes.

The number of nasal mucosal histopathology

There was a difference between lymphocyte, neutrophil, and eosinophil count in the control and treatment group, pre- and post-treatment, but it was not statistically significant with p-value >0.05. Post-treatment neutrophil count of the nasal mucosa in the treatment group was lower compared

to neutrophil count prior to treatment. The neutrophil count of the nasal mucosa in the treatment group was lower compared to the control group.

Neutrophils are the most abundant inflammatory cells infiltrating new wounds, and its main function is to dispose debris and prevent infection.[12] Although neutrophil has its role in preventing infection, persistent neutrophils presence in the wound will halt the wound-healing process. This will cause the acute wound to progress into a chronic wound.[13]

FESS is performed by creating as minimally invasive as possible towards the nasal mucosa. The healing process after the surgery is generally divided into two periods, which are the early period (early week), and the late period (up to 6 months). The early period is mainly affected by the surgery itself, post-surgery therapy, and the pathology that occurs; followed by the wound-healing process. Hoseman et al. quoted by Watelet et al.[14] stated that the healing process occurs in 4 phases: 1. Formation of hematic crusts (up to 10 days); 2. Lymphedema (up to 30 days); 3. Mesenchymal regeneration (up to 3 months); and 4. Crusts formation (after 3 months). Studies by Watelet et al.[15] elucidated that macrophages and neutrophils of the nasal mucosa were increased compared to the control group 1 month post FESS.

As the main component of extracellular matrices, HA is the primary structure in the wound-healing process.[16] In a study performed on mice, HA prevented elastin damage through elastase, and modulation of the secretion of neutrophil elastase. HA also could reduce the action of neutrophil elastase and metaloelastase in humans.[17] Study performed by Gelardi et al.[18] (2013) found a significantly reduced number of neutrophils in the group which received HA treatment, compared to the control group. In a study by Manuelle et al.[19] discovered that in children with recurrent respiratory tract infection who received HA treatment gained beneficial effect, which was a decrease in neutrophil number. Another study by Ciafalo et al.[20], in patients with acute rhinosinusitis who received HA therapy and nasal irrigation, could significantly decrease the number of neutrophils in cytological examination.

This study was in accordance with those studies, which showed that there was a decrease in nasal mucosal neutrophil count in the group receiving HA compared to the control group post FESS. HA could repair the damaged nasal mucosa and modulate cellular function, both related to nasal pathology and due to the FESS procedure. Although FESS was not aggressive nor destructive, nasal mucosa would still be damaged, which would be followed by post-surgical wound healing. The remodelling potency of HA is the basic element for post-FESS therapy. Based on this result, it could be understood that topical HA applications could protect sino-nasal epithelial from nasal mucosal damage in the post-surgery inflammatory process.

The number of eosinophils in the nasal mucosa after surgery in the treatment group and control group in this study was inversely related to the neutrophil count. There was an increase in the eosinophil count of the treatment group and a decrease in the control group, after the intervention compared to before the intervention. This result differed from the study performed by Gelardi et al.[18] (2013), which obtained a decrease in eosinophil count in the treatment group compared to the control group. Meanwhile in the study by Manuelle et al.[19] that was performed on children with recurrent respiratory tract infection treated with HA, the result did not show any effect of HA.

HA can increase GM-CSF, TGF-B, and ICAM-1 expression through eosinophil, by increasing the endurance of eosinophil. Interaction between HA and eosinophil contributes to inflammatory regulation and remodelling of the respiratory tract. HA

contributes to long-term eosinophil endurance by increasing GM-CSF production. HA invokes GM-CSF mRNA stabilization in eosinophil.[21] This is in accordance with the result of this study, which was an increase in eosinophil count in the group receiving HA therapy.

In accordance with the neutrophil count, the lymphocyte count in the control group and treatment group before and after intervention decreased, though not statistically significant with p>0.05. A study by Talaat et al.[22] in CRS patients who underwent FESS showed a significant increase of the T lymphocyte (CD3, CD4, and CD8) in patients who did not give clinical and endoscopic response after FESS. Grever et al.[23] also found a significant increase in T lymphocyte CD3, CD4, and CD8 in frozen section examination of the inferior turbinate of CRS patients.

T lymphocyte has a role in the pathophysiology of persistent inflammation in CRS. Based on the study by Talaat et al.[22], the dominant T lymphocyte increased in persistent chronic inflammation of the nasal and paranasal sinuses was CD4 subset. Lymphocyte affects immunological response through cell contact and cytokines release. An increase in type 1 T-helper (Th-1) and type 2 T-helper (Th-2), cytokines produced by lymphocytes could cause nasal mucosal damage, and increase nasal polyposis inflammation.[24]

According to literature and some studies that had been discussed above, it could be understood that high lymphocytes in the nasal mucosa gave unfavorable prognostic prediction in FESS. Lymphocyte and cytokines products could cause nasal mucosal damage. Thus, the result of this study showed that topical HA application post-FESS could decrease the nasal mucosal damage after the surgery, as can be seen from the lowered lymphocyte count after the intervention.

Level of Eosinophil-Lymphocyte Ratio and Neutrophil-Lymphocyte Ratio in the nasal mucosal histopathology

Eosinophil-to-lymphocyte ratio (ELR) of the nasal mucosa in the treatment group before and after the intervention was statistically significant with p <0.05, while the neutrophil-to-lymphocyte ratio (NLR) of the nasal mucosa was different but not statistically significant with p >0.05. Both ELR and NLR of the nasal mucosa in the treatment and control group before and after intervention were decreased.

NLR in blood has been raising interest, since it can be easily counted from complete blood count that has been routinely performed, and NLR nowadays had been used as a good indicator to find out the inflammatory status. ELR in blood nowadays has been used as a potential marker of systemic reaction due to an ongoing inflammatory process.

Boztepe et al.[25] (2016) compared NLR prior to the surgery from 88 CRS patients with a recurrent polyp and 24 patients with polyp but no recurrence. The result obtained was higher NLR in patients with a recurrent polyp. In another study, out of 158 CRS with polyp patients, Yenigun et al.[26] reported that patients with recurring polyposis had a significantly higher pre-surgery NLR and ELR compared to the ones without recurrence. Studies by Brescia et al.[27] showed that out of 240 CRS patients with polyp undergoing Endoscopic Sinus Surgery (ESS) followed for 12 months, also identified a significantly higher NLR and ELR before surgery compared to the patients without recurrence.

A sub-cohort study by Giuseppe et al.[28] on CRS with polyp patients, statistical analysis showed that average blood ELR and NLR were significantly lower after surgery compared to before. In accordance with the said study, the results of this study also showed a low average of nasal mucosal NLR and ELR

after the intervention. A significant result was obtained in average nasal mucosal ELR in the treatment group after the intervention, compared to before treatment. To the best of our knowledge, there had been no reports of NLR and ELR of the nasal mucosa. Therefore, the present study was the first to compare the NLR and ELR of the nasal mucosa before and after treated with HA.

The limitation of this study was that the evaluation was only carried out until 4 weeks. While the wound healing process is still going until 6 months. We also did not differentiate the subjects based on the history of allergy or without allergy.

In conclusion, this study had shown that topical HA could positively modulate the inflammatory response. Interestingly, there seemed to be inversed trends demonstrated by inflammatory cells count. When the number of neutrophils was decreased, eosinophil would be increased. However, there was no statistically significant difference found between the two groups, except for ELR in the treatment group.

This research was a preliminary study describing inflammatory cells on nasal mucosal wound healing treated with HA as adjuvant therapy after FESS. Further study needs to be done with longer follow up until the wound healing process is ended so that we can figure out the inflammatory response in nasal mucosa more clearly.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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