The IMPACT of chronic rhinosinusitis on olfaction (immunohistochemical study)

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Introduction
Chronic sinusitis is considered one of the most important causes of olfactory dysfunction, and this is attributed to the obstructive effect of swollen mucosa and nasal polyps or inflammatory process in the olfactory mucosa.

Patients and methods
This study enrolled 60 patients [20 chronic rhinosinusitis with nasal polyps (CRSwp), chronic rhinosinusitis without nasal polyps (20 CRSsp), and 20 control] and used nestin antibody, a marker found in olfactory mucosa and directly related to function, to prove the etiology of olfactory dysfunction in CRS.

Results
It was found that nestin staining is decreased in both cases groups, but on the contrary, patients with CRSwp have more olfactory dysfunction than patients with CRSsp. This means that the inflammatory process is strongly present in both case groups; however, the presence of polyps increases olfactory function loss.

Conclusion
This study led to an important conclusion that both obstructive and inflammatory causes contribute to olfactory dysfunction in patients with CRS.

Keywords:
anosmia, chronic sinusitis, hyposmia, nestin, olfaction

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Introduction

Chronic rhinosinusitis (CRS) is the most common cause of olfactory dysfunction, accounting for 14–30% of cases. Olfactory dysfunction in cases of CRS, was explained by a conductive olfactory loss, caused by swollen or hypertrophied nasal mucosa or nasal polyps, but this hypothesis failed to be proven, as there is only a slight correlation between nasal resistance and the degree of olfactory dysfunction [1].

Other studies have shown that the olfactory disturbance can be explained by inflammatory process in the olfactory cleft, proven by biopsies of the olfactory neuroepithelium, which revealed inflammatory changes in the nasal mucosa [2].

Nestin is an intermediate filament protein present in the basal region of the adult olfactory neuroepithelium in the zone that supports the ongoing neurogenesis in adults [3]. Nestin immunoreactivity was found to be related to olfactory function and is downregulated with loss of olfactory function [4].

Patients and methods

This study was approved by faculty of Medicine Ain Shams university research ethics committee (FMASU REC) which is organized and operated according to guidelines of the International Council on Harmonization (ICH) and the United States Office for Human Research Protections and operates under Federal Wide Assurance No. FWA 000017585.

This study included three patient groups:
(1) Study group 1: 20 patients with CRS without nasal polyps (CRSsp)
(2) Study group 2: 20 patients with CRS with nasal polyps (CRSwp)
(3) Control group: 20 patients complaining of nasal obstruction due to other nasal pathology.

Inclusion criteria

All adult patients with CRS diagnosed clinically according to Fokkens *et al.* (2012), and radiologically according to Lund-Mackay computed tomography (CT) scoring were included.

Exclusion criteria

Acute sinusitis, invasive acute or chronic fungal sinusitis, and chronic granulomatous inflammations were the exclusion criteria.

Methods

All patients were subjected to the following: full history taking, full otorhinolaryngologic examination, and evaluation of smell according to Alexander and Rand test, which was interpreted based on ground coffee smell and will take the following scores: (a) cannot smell, (b) smell something, and (c) recognize coffee [5].

CT scan of paranasal sinus was done, and grading of CRS was according to Lund-Mackay CT scoring.

Biopsy specimens were obtained from olfactory mucosa about 1–2 mm^3 from the superior aspect of the middle turbinate or the opposed septum during functional endoscopic sinus surgery or during septoplasty. Specimens were fixed in 10% formalin, paraffin embedded, sectioned, and processed for routine H and E stain and immunohistochemical staining with nestin antibody (Gene Tex, Catalog number: GTX630201). The immunohistochemistry was performed using the peroxidase-antiperoxidase method in combination with 3-3'-diaminobenzidine. The slides were examined microscopically to assess the histopathological changes in the olfactory mucosa.

Results

Study population

This study enrolled 60 patients divided into three groups: cases CRSwp, cases CRSsp, and controls. Age of the patients ranged from 8 to 60 years, with mean age 36 years.

There was slight preponderance toward male sex (55%), with females representing 45%, among patients with CRSsp, whereas the opposite was found in patients with CRSwp, with 45% males and 55% females.

Olfaction

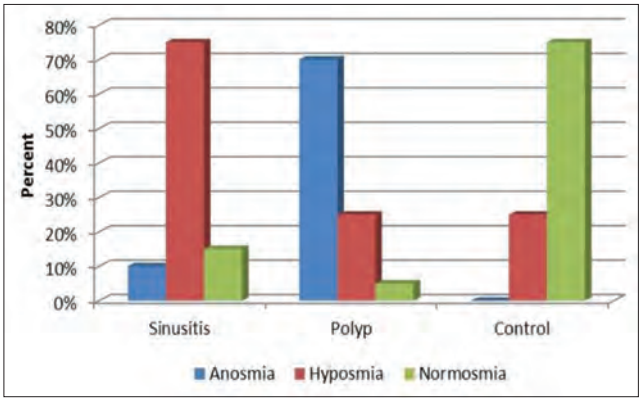
Study group 1 (CRSsp) was found to have hyposmia in 75% of cases, anosmia in 15% of cases, and 10% normosmia. However, in study group 2 (CRSwp), anosmia was found in 70% of cases, hyposmia in 25% of cases, and 5% normosmia. The control group was found to have 75% normosmia and 25% hyposmia cases (Fig. 1).

Pathological changes

Control group showed strong nestin staining in 90% of cases, with unremarkable other pathological changes (Table 1 and Fig. 2).

Study group 1 showed that 75% of studied cases had partial erosion of epithelium, and 55% of the cases showed moderate inflammatory cells infiltration in the lamina propria and mild intraepithelial cellular infiltrate in 45% of cases (Fig. 3). Moreover, it showed degeneration of epithelium in 20% of cases, squamous metaplasia in two cases (Figs. 4 and 5), and mucinous metaplasia in three cases. Regarding nestin staining,

Figure 1



comparison of olfaction between the three groups.

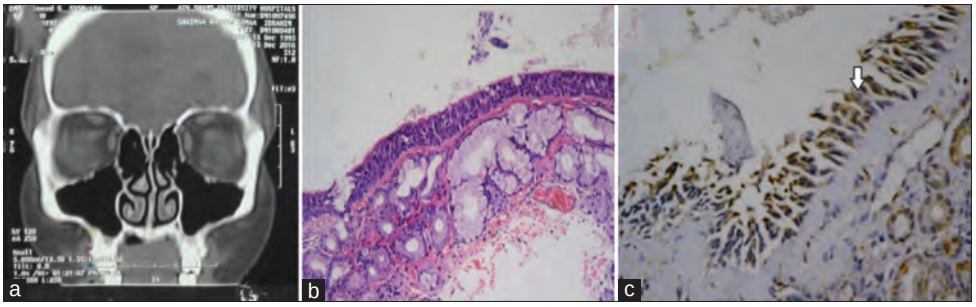
Table 1 Nestin staining in control group

	n (%)
Nestin stain	
Mild	2 (10.0)
Strong	18 (90.0)

Table 2 Histopathological findings in study group 1

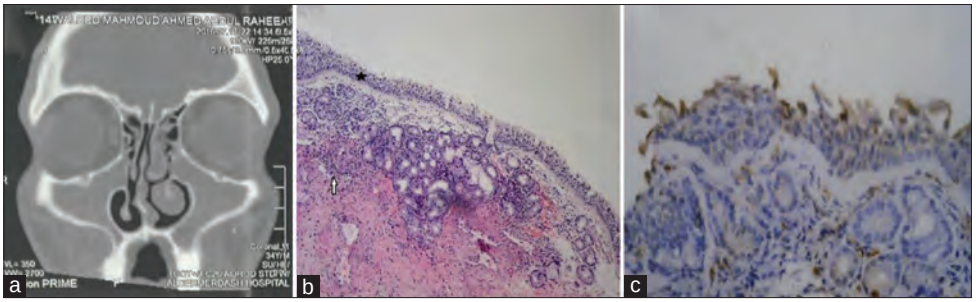
	n (%)
Nestin stain	
Negative	9 (45.0)
Mild	8 (40.0)
Moderate	3 (15.0)
Erosion of epithelium	
No	3 (15.0)
Partial	15 (75.0)
Complete	2 (10.0)
Degeneration of epithelium	
No	3 (15.0)
Mild	6 (30.0)
Moderate	7 (35.0)
Severe	4 (20.0)
Inflammatory cells in lamina	
Mild	4 (20.0)
Moderate	11 (55.0)
Severe	5 (25.0)
Inflammatory cells intraepithelial	
No	10 (50.0)
Mild	9 (45.0)
Moderate	1 (5.0)
Squamous metaplasia	
No	18 (90.0)
Yes	2 (10.0)
Mucinous metaplasia	
No	17 (85.0)
Yes	3 (15.0)

Figure 2



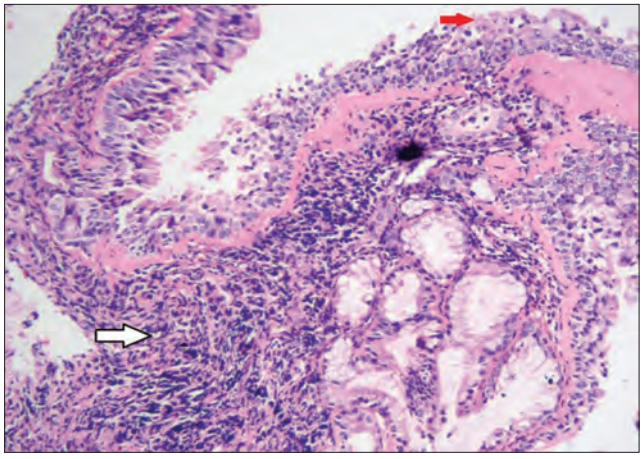
A control case (patient with hypertrophied inferior turbinates). A: CT paranasal (coronal cut), B: Normal olfactory epithelium (H&E x100), C: normal olfactory mucosa with strong nestin staining (white arrow) (Nestin x200).

Figure 3



A case of CRS without polyps, A: CT paranasal (coronal cut), B: H&E showing mild intraepithelial lymphocytic infiltrate (star),and mild subepithelial inflammatory cellular infiltrate(white arrow) (H&E x100), and preserved olfactory epithelium, C: reduced (moderate) staining of olfactory epithelium with nestin (Nestin x400).

Figure 4



A case of CRS showing marked inflammatory cells infiltrate in subepithelial tissue(white arrow), and focal squamous metaplasia of surface epithelium(red arrow) (H&E x200).

40% of cases showed mild staining with nestin, 15% showed moderate staining, whereas 45% did not stain with nestin (Table 2).

Study group 2 showed that most studied cases had partial erosion of epithelium (95%) (Fig. 6) and more than half of the cases showed moderate inflammatory cells infiltration in the lamina propria (60%), and 55% of cases had intraepithelial inflammatory cell infiltrate. Moreover, the group showed degeneration of epithelium in 25% of cases, and mucinous metaplasia in two cases (Fig. 7). Moreover, the group showed 60% were nestin stain negative (Table 3 and Figs. 8 and 9).

Comparison among three study groups regarding personal and clinical characteristics

There was no significant difference among the three study groups regarding personal characteristics; however, a significant difference among the three study groups was found regarding olfaction (Table 4).

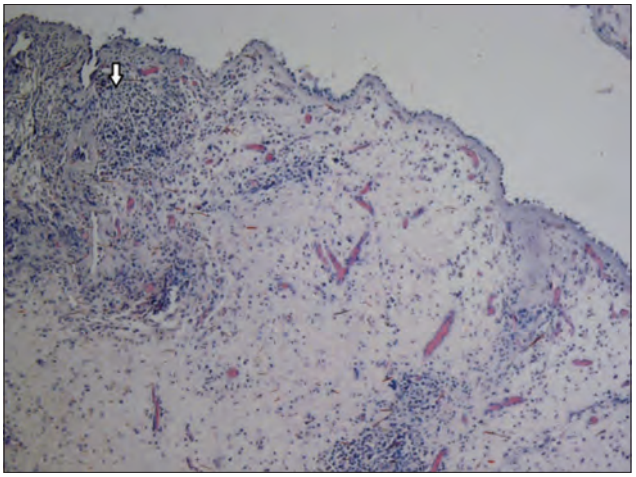
Comparison between sinusitis and polyp groups regarding personal and clinical characteristics

There was no significant difference between sinusitis and polyp groups regarding personal characteristics; however, a significant difference between the two study groups was found regarding olfaction (Table 5).

Comparison among three study groups regarding nestin stain

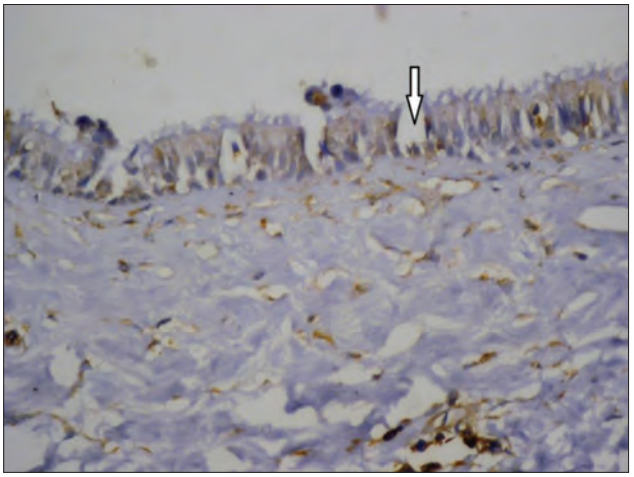
There was a highly statistically significant difference among the three groups regarding nestin stain. However, on comparing the two case groups, the difference was nonsignificant (Tables 6, 7 and Fig. 10).

Figure 5



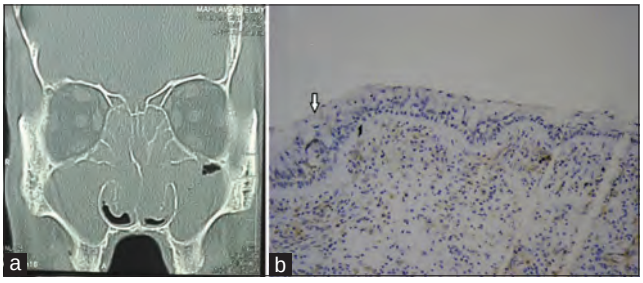
A case of CRS showing degeneration of surface epithelium. There are edema,congestion and lymphoid follicle formation in subepithelial tissue (white arrow) (H&E x100).

Figure 6



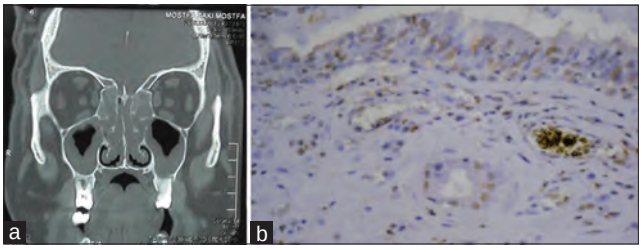
A case of CRS showing focal erosion of olfactory mucosa(white arrow) and moderate nestin staining (Nestin x400).

Figure 7



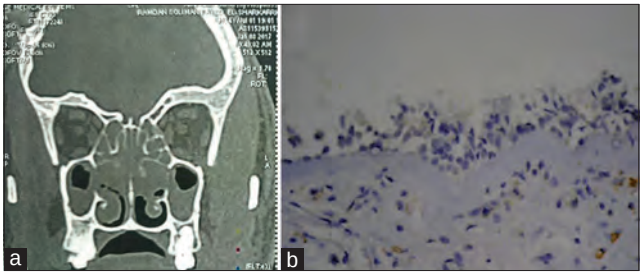
A case of CRS with polyps, A: CT paranasal (coronal cut), B: negative staining with nestin and mucinous metaplasia of surface olfactory epithelium (white arrow) (Nestin x400).

Figure 8



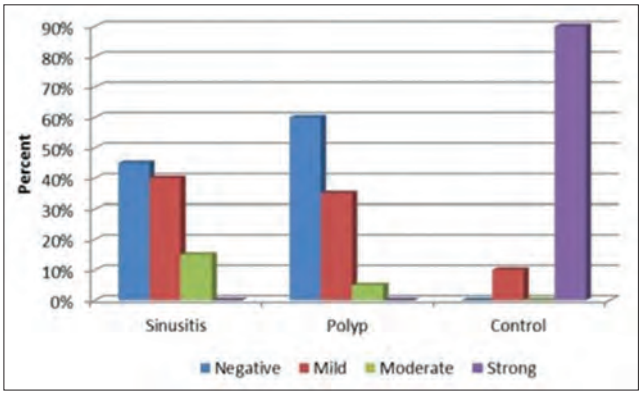
A case of CRS with polyps, A: CT paranasal (coronal cut), B: mild staining of olfactory mucosa with nestin (Nestin x400).

Figure 9



A case of CRS with polyps, A: CT paranasal (coronal cut), B: degenerated olfactory epithelium and mild staining with nestin (Nestin x400).

Figure 10



Comparison between three study groups as regards nestin stain.

Relation between olfaction and radio score and relation between nestin stain and radio score

The results revealed a significant relation between olfaction and radiological score, whereas there was a nonsignificant relation between radiological score and nestin stain (Tables 8 and 9).

Discussion

Generally CRS has an impaction olfactory function. This occurs through different pathogenesis. Most previous studies proved that this occurs either through obstructive pathway or inflammatory pathway.

Yee *et al.* [6], performed a study on 54 patients to prove the presence of inflammatory changes in olfactory neuroepithelium using histopathology and immunohistochemical studies and proved inflammatory changes to be the cause of olfactory dysfunction in CRS.

Kern *et al.* [7] also stated that pathogenesis of olfactory dysfunction is apoptotic cell death in olfactory sensory neuron of olfactory epithelium, and this is the explanation of olfactory dysfunction whatever the cause.

Rombaux *et al.* [1] made a review of literature and attributed olfactory dysfunction to invasion of olfactory epithelium by inflammatory cells, especially eosinophils, and stated that patient with nasal polyps have better olfactory functions, as odor reaches the olfactory mucosa through the retronasal route.

Table 3 Histopathological findings in study group 2

	n (%)
Nestin stain	
Negative	12 (60.0)
Mild	7 (35.0)
Moderate	1 (5.0)
Erosion of epithelium	
Partial	19 (95.0)
Complete	1 (5.0)
Degeneration of epithelium	
Mild	8 (40.0)
Moderate	7 (35.0)
Severe	5 (25.0)
Inflammatory cells in lamina	
Mild	4 (20.0)
Moderate	12 (60.0)
Severe	4 (20.0)
Inflammatory cells intraepithelial	
No	8 (40.0)
Mild	11 (55.0)
Moderate	1 (5.0)
Squamous metaplasia	
No	20 (100.0)
Mucinous metaplasia	
No	18 (90.0)
Yes	2 (10.0)

Banglawala *et al.* [8], conducted a meta-analysis study on olfaction in patients with CRS and reached an outcome that loss of function is multifactorial, where both mechanical and inflammatory causes are involved.

Sanchez *et al.* [9], conducted a study on 33 patients, where they assessed olfaction in patients with CRS before surgery to prove the presence of olfactory dysfunction, and stated a hypothesis that loss of function is multifactorial depending on presence of nasal polyps and inflammation of epithelium.

Huart *et al.* [2] put forward a hypothesis that olfactory dysfunction is owing to inflammation in olfactory epithelium, and mechanical obstruction has a synergistic effect toward the increase in the function loss.

This study was done on 40 patients divided into two groups (CRSwp and CRSsp), who were compared with 20 control patients. It was based on the examination of an immunohistochemical marker (nestin), to proven that its expression is directly correlated with olfactory function, as it was found in previous studies that nestin immunoreactivity is clearly present in the sustentacular cell layer of normosmic patients, reduced in the OE of patients with hyposmia, and in the OE of anosmic patients, it was found to be noticeably reduced or lost [4].

Results showed that inflammatory changes and reduction of nestin expression were found in both CRSwp and CRSsp groups, to be markedly significant compared with the control group ($P < 0.001$), but with nonsignificant difference between each other ($P = 0.543$) (Tables 6 and 7), and this proves that the inflammatory process is found in both groups and is responsible for the loss of function.

On the contrary, it was found also that the degree of olfactory dysfunction is not the same in both cases groups (CRSwp and CRSsp), and there is a high significant difference in loss of function between them ($P < 0.0001$) (Table 5), as in CRPsp, it was found that most cases were hyposmic, whereas in CRSwp, it was found that most cases were anosmic.

Table 4 Comparison between three study groups regarding personal and clinical characteristics

	Group (mean±SD)			*P*	Significance
	Sinusitis	Polyp	Control		
Age	36.00±13.78	31.00±12.99	27.40±6.59	0.158	NS
Sex [*n* (%)]					
Male	11 (55.0)	9 (45.0)	8 (40.0)	0.626	NS
Female	9 (45.0)	11 (55.0)	12 (60.0)		
Olfaction [*n* (%)]					
Anosmia	2 (10.0)	14 (70.0)	0	0.001	HS
Hyposmia	15 (75.0)	5 (25.0)	5 (25.0)		
Normosmia	3 (15.0)	1 (5.0)	15 (75.0)		

HS, highly significant; NS, nonsignificant.

Table 5 Comparison between sinusitis and polyp groups as regard personal and clinical characteristics

	Group (mean±SD)		*P*	Significant
	Sinusitis	Polyp		
Age	36.00±13.78	31.00±12.99	0.245	NS
Sex [*n* (%)]				
Male	11 (55.0)	9 (45.0)	0.752	NS
Female	9 (45.0)	11 (55.0)		
Olfaction [*n* (%)]				
Anosmia	2 (10.0)	14 (70.0)	0.0001	HS
Hyposmia	15 (75.0)	5 (25.0)		
Normosmia	3 (15.0)	1 (5.0)		

HS, highly significant; NS, nonsignificant.

Table 6 Comparison between three study groups as regard nestin stain

	Group [*n* (%)]			*P*	Significant
	Sinusitis	Polyp	Control		
Nestin stain					
Negative	9 (45.0)	12 (60.0)	0	0.001	HS
Mild	8 (40.0)	7 (35.0)	2 (10.0)		
Moderate	3 (15.0)	1 (5.0)	0		
Strong	0	0	18 (90.0)		

HS, highly significant.

Table 7 Comparison between sinusitis and polyp groups groups as regard nestin stain

	Group [*n* (%)]		*P*	Significant
	Sinusitis	Polyp		
Nestin stain				
Negative	9 (45.0)	12 (60.0)	0.543	NS
Mild	8 (40.0)	7 (35.0)		
Moderate	3 (15.0)	1 (5.0)		

NS, nonsignificant.

Table 8 Relation between each of olfaction and radio score

	Radio score		*P*	Significant
	Mean±SD	Median		
Olfaction				
Anosmia	15.00±5.54	16.00	0.016	S
Hyposmia	11.15±6.03	10.00		
Normosmia	6.50±1.91	6.00		

S, significant.

Table 9 Relation between nestin stain and radio score

	Radio score		*P*	Significant
	Mean±SD	Median		
Nestin stain				
Negative	11.67±6.13	10.00	0.751	NS
Mild	13.27±6.22	15.00		
Moderate	11.25±6.34	11.00		

NS, nonsignificant.

These results led us to a new hypothesis that both obstructive and inflammatory causes share in the olfactory impairment. In cases of CRPsp, only the inflammatory changes play the role causing only hyposmic changes, whereas in CRSwp, the presence of obstructive agent, which is the polyps, adds on the loss of function, leading to anosmic changes.

These results opposed Yee *et al.* [6], and Kern *et al.* [7], as they ignored the obstructive element that was proven by these results, and also this study had upper hand by using nestin protein marker, which is directly related to olfactory function.

Moreover, this study was in contrast to Rombaux *et al.* [1], who stated that patients with polyps have better olfactory function and dysfunction only related to inflammatory causes, and these results opposed this hypothesis by the findings of highly significant difference in olfactory function between the two study groups (CRSsp and CRSwp) ($P < 0.0001$).

On the contrary, these results agreed with a meta-analysis by Sanchez *et al.* [9], and the study by Banglawala *et al.* [8], although they did not use any histopathological studies to prove this hypothesis as this study did.

Moreover, this study came in close agreement with the hypothesis of Huart *et al.* [2], but had the upper hand by proving this hypothesis by using immunohistochemical studies with nestin protein marker.

Therefore, this study is one of the very few studies that proved multifactorial hypothesis using immunohistochemical studies with nestin protein marker.

In this study also the results showed atrophy of epithelium in 20–25% of cases and showed squamous and mucinous metaplasia in seven cases. This highlights the severity of inflammation, which can cause metaplasia and act as a precancerous predictor.

Conclusion

This study led us to an important conclusion that both obstructive and inflammatory causes contribute together to olfactory dysfunction in patients with CRS.

Therefore, if we aim to treat olfactory impairment caused by CRS. The treatment plan must include both surgical and medical plans, aiming to overcome both causes of dysfunction.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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