e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(8); 190-197

Original Research Article

An Evaluation and Analysis of Immune Markers in Patients with Chronic Rhinosinusitis

Hanish Haneefa[1], Nazeem Abdul Majeed[2]

[1]Associate Professor, Department of ENT, Kannur Medical College, Anjarakandy, Kannur, Kerala

[2]Associate Professor, Department of ENT, Kannur Medical College, Anjarakandy, Kannur, Kerala

Received: 05-06-2022 / Revised: 18-06-2022 / Accepted: 20-07-2022

Corresponding author: Dr Hanish Haneefa

Conflict of interest: Nil

Abstract

Background: Chronic rhino sinusitis (CRS) is a common inflammatory disease resulting in marked decrease in quality of life of patients and affected their socio-economic life. Multiple factors like inborn immunity of the airway epithelium, reduced barrier function, altered mucociliary mechanism, and fabrication of plenty of antimicrobial peptides played important roles. Followed by activated involvement of eosinophils, mast cells, and innate lymphoid cells (ILCs) contributed to the chronic inflammatory changes and directly activated adaptive immune cells like T and B cells. Studies are required to identify such specific immune factors which drive the CRS pathogenesis process and this study is an attempt in that direction.

Aim: To find the role of immune markers in the pathogenesis of the chronic inflammation in chronic rhino sinusitis (CRS) and to identify other markers of immune system pathways in patients with CRS.

Materials: 83 patients diagnosed with CRS based on the Clinical features and X-ray and CT scan findings were divided into two groups; CRSsNP- 42 patients (50.60%) and CRSwNP- 41 (49.39%) patients.

Results: The mean levels of secreted IL-6, 10, 13, 21 and IFN- γ of both the groups of CRSwNP-41 patients and CRSsNP-42 patients' tissue levels correlated well ($p > 0.05$ for all). CRSwNP patients had mean tissue levels of IL-2, 4, 5, 7, 12, 17 and 22 greater than mean tissue levels of patients with patients CRSsNP.

Conclusions: The two types of CRS: CRSsNP and CRSwNP are heterogeneous diseases at molecular level as the values of cytokines liberated due to respective inflammation are different. Hence the inflammatory state of CRS was also highly heterogeneous, with mixed profiles of type 1, 2 and 3 inflammations seen within classical CRSsNP and CRSwNP phenotypes. Estimation of cytokines levels is emerging as an important diagnostic and prognostic tool in the management of CRS disease.

Keywords: Chronic Rhino Sinusitis (CRS), Inflammation, Adaptive Immunity, Mucociliary Mechanism, Facial Pain and Innate Immunity.

Background

Chronic rhino sinusitis (CRS) is a disease of nose and paranasal sinuses, associated with marked morbidity and burden on socio-economic life of the patients [1]. In spite of its burden on the community only few studies have focused on its prevalence, nature of the diseases and actual pathological mechanisms and immune factors involved in causing CRS [2]. CRS clinically presents in two forms: CRS without nasal polyps (CRSsWP) and CRS with nasal polyps (CRSwNP) [3]. Both these forms do not represent the two ends of the same disease spectrum but are caused by unique inflammatory mechanisms [3]. The published data was focused on CRSwNP pathogenesis alone or CRS in general and hence a large data is available on it. But the data did not reflect on any immune specific factors or properties associated with a particular subgroup of CRS [4]. Hence only data on distinct inflammatory characteristics associated with CRSwNP were available published but very few on the unique inflammatory signature profiles associated with CRSsNP [5]. Thus, this review will highlight the more recent advances in our understanding of the mechanisms that underlie CRS pathogenesis, and will focus mainly on CRSwNP. Chronic inflammation of sinonasal mucosa lasting for more than 12 weeks with symptoms of nasal obstruction, nasal discharge, pain in the face and head region loss of sense of smell was defined s CR [6] CRS was treated by using antibiotics, nasal and oral decongestants and in few cases with steroids [6]. When medical treatment did not alleviate symptoms functional endoscopic sinus surgery was undertaken [7]. There are no population based epidemiological studies to specify the proportion of cases requiring surgery or repeat surgeries [8]. The prevalence of CRSsNP cases is greater than the cases of CRSwNP as per few studies and some other studies reported equal prevalence [9].The prevalence of repeat surgeries was more common in CRSwNP than in CRSsNP according to the literature available [3]. Few studies showed predominance of males suffering from CRSwNP than females [4] but women tend to have severe inflammation and required more surgeries than men [6]. The data further revealed that the pathogenesis of the two forms of CRS was likely distinct, and suggest that CRSsNP is more associated with specific infections, while CRSwNP is more associated with inflammatory airway disease [2]. Epithelial cells of sinonasal disease mucosa from CRSwNP subjects were shown to have defects in the ability to form tight junctions [11]. In addition elevated type 2 cytokines levels in CRSwNP was thought to produce a diminished barrier function of these cells [11]. It was observed that patients with CRSwNP have elevated epithelial anion transporter Pendrin in their mucosal polyps which increased mucus production and impaired mucociliary clearance [12]. Pendrin production was increased by the type 2 inflammatory cytokines IL-4 and IL-13 [12]. Recently few studies have shown bitter and sweet taste receptors T2R38 in the mucociliary cells and the bitter was found to regulate the ciliary beat frequency in airway epithelial cells [13]. Cohen and colleagues being first to identify these receptors also found that polymorphism in T2R38 gene resulted in non-functional taste receptors [14]. This polymorphism was observed in the patients who did not respond to medical

treatment of CRS but required surgical intervention [15]. Further follow up studies revealed that the bitter receptors could be controlled negatively the T1R family of sweet taste receptors [16], Hence the increased glucose concentration in the mucus from CRS patients [17].

T2R38 bitter taste receptors were proved to be associated with a decreased capacity of airway epithelial cells to kill bacteria [18]. Similarly production of innate antimicrobial peptides were also reduced and produced defectively, denying the function of normal airway epithelial cells to protect the underlying tissue mucosa from harmful microbes and antigens. The defects in expression of other innate antimicrobials in CRSwNP included were S100A7, S100A8/A9, and PLUNC [19]. Due to this large numbers of immune effector cells are found elevated in CRS, particularly in CRSwNP. They include eosinophils, neutrophils, basophils, mast cells, type 2 macrophages, and group 2 innate lymphoid cells (ILC2s) [20]. In older teachings the CRSsNP cases were described as neutrophilic and CRSwNP cases as eosinophilic; however it was observed that neutrophils were elevated in both types of CRS cases, but it was not evident whether eosinophils were elevated in CRSsNP cases [21]. Eosinophils are known to produce type 2 inflammatory response in nasal polyps of CRSwNP and cause elevation of eosinophil chemo attractants and cytokines like: eotaxins and IL-5 [22]. Recently a new role of eosinophils was unrolled by which they promote plasma cell survival and antibody production and the activation of T cells and the antibodies produced by them [23]. Increased production of mucus in CRSwNP patients was proved to be due to the secretion of tryptase, carboxypeptidase A3, and chymase by the mast cells [24]. Mast cells of nasal glands produce chymase unlike the nasal mucosal mast cells and the chymase is a stimulator of mucus secretions in CRSwNP patients [25]. Lymphocytes in the nasal mucosa with chronic type 2 inflammation produce type 2 cytokines like IL-5 and IL-13 which are activated by cytokines from epithelial cells such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) [26]. Spits and colleagues [27] have shown ILC2s in highly elevated levels in nasal polyp tissues. There is an increased adaptive immune cell in the type CRSwNP polyps. $CD3^+$ T cells have been found to be elevated in both CRSsNP and CRSwNP tissues [28].

Materials

82 patients with diagnosis of CRS were included in the study after obtaining an ethical committee clearance for the Institute. An ethics committee approved consent form was used for the study.

Inclusion Criteria: Patients aged 18 years and above were included. Patients of both genders were included. Patients with clinical features of nasal obstruction, chronic nasal discharge, facial congestion, facial pain, facial pressure, facial fullness, and decrease in sense of smell and Presence or absence of nasal polypi on CT scan or nasal endoscopy were included.

Exclusion criteria: Patients with history of primary or secondary immune disorders were excluded. Patients with Cystic fibrosis were excluded. Patients with Primary ciliary dyskinesia were excluded. Patients with pregnancy were excluded. Patients with history of smoking in the last 12 weeks were excluded. SNOT-22 scores [29] greater than 21 (to exclude those currently in exacerbation). Patients with history of intake of oral corticosteroids in the past 4 weeks were excluded. Patients with history of parenteral steroids therapy in the past 4 weeks were excluded. Demographic data, medical co-morbidities, and history of smoking were elicited from the patients.

Method of Detecting immune markers and their concentrations: Polypoidal tissue from FESS operations, mucosal biopsies from CRS patients undergoing surgery or otherwise as a biopsy from the middle turbinate were collected. Tissue samples were immediately frozen and stored at −80 °C till further usage. About 40 mg of tissue was shifted to a Buffer AL extraction reagent (Quiagen) containing gentle-MACS C tube. Gentle-MACS homogenizer was used till the tissue was evenly suspended. It was centrifuged after mixing it 1.5-ml polypropylene at room temperature for 5 minutes. The supernatant fluid was collected and an aliquot removed and diluted twice with kit-provided buffer. This material was then assessed for levels of interleukin (IL)-2, -4, -5, -6, -7, -10, -12, -13, -17, -21, and -22, as well as interferon (IFN)-γ using a Luminex R multiplex kit (Human Premixed Multi-Analyte, R&D Systems, Minneapolis, MN) according to manufacturer protocols. Each sample was analyzed in triplicate. Statistical analysis: All the data was analyzed using the SPSS v.23.0 statistical package. All the data was non-normally distributed and compared using a Mann–Whitney *U*-test. A Chi-square and Fisher's exact test were used to determine whether the data were related. Binary logistic regression analysis was used to find the predictive values of cytokine levels for CRSwNP. Differences between groups were considered statistically significant at $p < 0.05$.

Results

83 patients were included in this study that was diagnosed with CRS based on the Clinical features and X-ray and CT scan findings. The study was conducted over a period of 12 months between March 2021 and February 2022. The subjects were divided into two groups; Group A consisting of 41 (49.39%) patients with CRSwNP type CRs and Group B: consisting of 42 (50.60%) patients with CRSsNP type of CRS. The demographic details, gender distribution and co-morbid conditions were tabulated in Table 1. The mean ages and ratios of females: males in both groups were similar. The frequency of allergy or asthma was significantly higher in the CRSwNP group. Than in the CRSsNP group; (p- value less than 0.05) (Table 1). A total of 27.27% of patients in the CRSwNP group and 38.09% of patients in the CRSsNP group gave history of smoking which was not significant.

Table 1: Showed the demographic features and co-morbid conditions of the two groups (n-83; Group A-41, Group-42).

Variable	Group A CRSwNP-41	Percentage	Group B CRSsNP-42	Percentage	P value
Number	41	49.39	42	50.60	0.753
Mean Age	47.55±3.75	--	41.65±5.10	--	0.941
Male	24	54.54	21	50	0.845
Female	17	41.46	18	42.85	0.614
Allergy					
Yes	14	34.14	03	42.85	0.001
No	30	73.17	39	07.14	
Asthma					
Yes	16	39.02	04	09.52	0.001
No	28	68.29	38	47.61	
Smoking					
Yes	12	27.27	16	38.09	0.093

No	32	72.72	26	61.90	
Diabetes					
Yes	10	24.39	11	26.19	0.851
No	34	82.92	31	73.80	
Drug allergy					
Yes	13	31.70	17	35.71	0.061
No	21	51.21	25	59.52	

The mean levels of secreted IL-6, 10, 13, 21 and IFN- γ of both the groups of CRSwNP-41 patients and CRSsNP-42 patients' tissue levels correlated well ($p > 0.05$ for all). CRSwNP patients had mean tissue levels of IL-2, 4, 5, 7, 12, 17 and 22 greater than mean tissue levels of patients with patients CRSsNP (Table 2).

Table 2: Showed the mean values of Interleukins (ILs) in both the groups (n-83; Group A-41, Group-42)

TYPES OF ILs	Mean values in CRSwNP-41	Mean values in CRSsNP-42	p- value
IL-6	189.1	229.8	0.912
IL-10	219.6	214.7	0.712
IL-13	148.63	153.3	0.478
IL-21	104.5	103.6	0.351
IFN-γ	1398.0	1376.5	0.140
IL-2	477.3	149.3	0.027
IL-4	189.3	69.6	0.019
IL-5	88.6	38.2	0.011
IL-7	59.9	27.5	0.033
IL-12	664.0	139.1	0.001
IL-17	98.1	59.0	0.001
IL-22	93.4	41.2	0.001

Discussion

In the present study 83 patients were included after strictly adhering to the inclusion and exclusion criteria and satisfying the SNOT-22 criteria. The tissues obtained from the surgical procedures and biopsies were obtained and processed for Interleukins with an intention to compare the two basic types of CRS that were CRSwNP and CRSsNP. A statistically significant difference in their mean values was observed in relation to the ILs: 2, 4, 5, 7, 12, 17 and 22 in the study. There was no significant difference between the two groups in regards to the ILs: 6, 10, 13, 21 and IFN- γ. Review of literature showed similar studies conducted to estimate concentrations of different cytokines to find the degree of type-2 chronic inflammation of the nasal and sinus mucosa in CRS. Zhao *et al* [30] demonstrated higher IL-5 concentrations in Nasal Polyps tissues than those in control tissues. Similar study by Kubato *et al* [31] in this study also the author had divided the CRS as CRSwNP and CRSsNP. But Bachert *et al* [32] in addition to IL-5 assayed eotaxin an Eosinophilic cationic protein which was an indicator of eosinophilic activator. Allen *et al* [33] demonstrated IL-5 levels in polyp tissue higher than in controls. Moore *et al* [34] from their study reported high levels of IL-10 in the nasal tissues and peripheral

blood of their Allergic rhinitis patients who were successfully treated with immunotherapy on long-term. In this study in contrast both the CRS with nasal polyp and only with CRS patients had similar mean tissue levels of IL-10. Adnan Ekinci and his co-author [35] in their study observed no difference in the tissue IL-10 levels, similar to the present study.

Molet SM *et al* studied the IL-17 levels from polyp tissue and mucosa of CRS without polyp's patients and reported statistically higher levels in polyp tissues. Dellacono FR *et al* observed in patients with Nasal Polyps a high concentration of IFN-γ levels than in nasal mucosa of CRS without polyps. They also suggested that IF -γ levels activated lymphocytes and eosinophils in the Nasal polypoidal mucosa. In this study IFN-γ levels were higher in both the groups. The limitations to this study were absence of comparisons between tissue cytokine levels and serum cytokines levels. There was no comparison of role of steroidal therapy on long term in either of the groups studied.

Conclusions

The two types of CRS: CRSsNP and CRSwNP are heterogeneous diseases at molecular level as the values of cytokines liberated due to respective inflammation are different. Hence the inflammatory state of CRS was also highly heterogeneous, with mixed profiles of type 1, 2 and 3 inflammations seen within classical CRSsNP and CRSwNP phenotypes. Estimation of cytokines levels is emerging as an important diagnostic and prognostic tool in the management of CRS disease. Identify inflammatory profiles of CRS in a clinical setting allowed precise and targeted treatment options. The identification of inflammatory profiles also allowed selection of targeted therapies.

References

1. Lange B, Thilsing T, Baelum J, Kjeldsen AD. The Sinonasal Outcome Test 22 score in persons without chronic rhinosinusitis. Clin Otolaryngol. 2016 Apr;41(2):127-30.
2. Farhood Z, Schlosser RJ, Pearse ME, Storck KA, Nguyen SA, Soler ZM. Twenty-two-item Sino-Nasal Outcome Test in a control population: a cross-sectional study and systematic review. Int Forum Allergy Rhinol. 2016 Mar;6(3):271-7.
3. Benninger MS, Ferguson BJ, Hadley JA, *et al*. Adult chronic rhinosinusitis: Definitions, diagnosis, epidemiology, and pathophysiology. Otolaryngol Head Neck Surg. 2003;129(suppl 3):S1–S32.
4. Meltzer EO, Hamilos DL, Hadley JA, *et al*. Rhinosinusitis: Establishing definitions for clinical research and patient care. Otolaryngol Head Neck Surg. 2004;131(suppl 6):S1–S62.
5. Van Zele T, Claeys S, Gevaert P, *et al*. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. Allergy. 2006; 61: 1280-1289.
6. Bhattacharyya N. Symptom outcomes after endoscopic sinus surgery for chronic rhinosinusitis. Arch Otolaryngol Head Neck Surg. 2004; 130:329–333.
7. Lund VJ. Surgical outcomes in chronic rhinosinusitis and nasal polyposis. Rhinology. 2006; 44:97.
8. Bhattacharyya N. Influence of polyps on outcomes after endoscopic sinus surgery. Laryngoscope. 2007; 117:1834–1838.
9. Ponikau JU, Sherris DA, Kern EB, *et al*. The diagnosis and incidence of allergic fungal sinusitis. Mayo Clin Proc. 1999; 74:877–884.
10. Shin SH, Ponikau JU, Sherris DA, *et al*. Chronic rhinosinusitis: An enhanced immune response to ubiquitous airborne fungi. J Allergy Clin Immunol. 2004; 114:1369–1375.

11. Seiberling KA, Grammer L, Kern RC. Chronic rhinosinusitis and superantigens. Otolaryngol Clin North Am. 2005; 38:1215–1236.
12. Van Zele T, Gevaert P, Holtappels G, *et al*. Local immunoglobulin production in nasal polyposis is modulated by superantigens. Clin Exp Allergy. 2007; 37:1840–1847.
13. Van Zele T, Gevaert P, Watelet JB, *et al*. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. J Allergy Clin Immunol. 2004; 114:981–983.
14. Douglas, J.E.; Cohen, N.A. Taste Receptors Mediate Sinonasal Immunity and Respiratory Disease. Int. J. Mol. Sci. 2017;18;437.
15. Adappa ND, Howland TJ, Palmer JN, Kennedy DW, Doghramji L, Lysenko A, *et al*. Genetics of the taste receptor T2R38 correlates with chronic rhinosinusitis necessitating surgical intervention. Int Forum Allergy Rhinol. 2013; 3(3):184–7.
16. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, *et al*. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. Rhinology Supplement. 2012; (23):3 p preceding table of contents, 1–17.
17. Comprehensive review of the latest definitions, practice standards and research on chronic rhinosinusitis.
18. Lee RJ, Kofonow JM, Rosen PL, Siebert AP, Chen B, Doghramji L *et al*. Bitter and sweet taste receptors regulate human upper respiratory innate immunity. J Clin Invest. 2014; 124(3):1393–405.
19. Seshadri S, Lin DC, Rosati M, Carter RG, Norton JE, Suh L, *et al*. Reduced expression of antimicrobial PLUNC proteins in nasal polyp tissues of patients with chronic rhinosinusitis. Allergy. 2012;67(7):920–8.
20. Hulse KE, Stevens WW, Tan BK, Schleimer RP. Pathogenesis of nasal polyposis. Clin Exp Allergy. 2015; 45(2):328–46.
21. Van Zele T, Claeys S, Gevaert P, Van Maele G, Holtappels G, Van Cauwenberge P, *et al*. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. Allergy. 2006;61(11):1280–9.
22. Stevens WW, Ocampo CJ, Berdnikovs S, Sakashita M, Mahdavinia M, Suh L, *et al*. Cytokines in chronic rhinosinusitis. Role in eosinophilia and aspirin-exacerbated respiratory disease. Am J Respir Crit Care Med. 2015;192 (6):682–94.
23. Chu VT, Berek C. The establishment of the plasma cell survival niche in the bone marrow. Immunol Rev. 2013;251(1):177–88.
24. Takabayashi T, Kato A, Peters AT, Suh LA, Carter R, Norton J, *et al*. Glandular mast cells with distinct phenotype are highly elevated in chronic rhinosinusitis with nasal polyps. J Allergy Clin Immunol. 2012;130(2):410–20.
25. Mahdavinia M, Carter RG, Ocampo CJ, Stevens W, Kato A, Tan BK, *et al*. Basophils are elevated in nasal polyps of patients with chronic rhinosinusitis without aspirin sensitivity. J Allergy Clin Immunol. 2014;133(6):1759–63.
26. Magri G, Miyajima M, Bascones S, Mortha A, Puga I, Cassis L, *et al*. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. Nat Immunol. 2014;15(4): 354–64.
27. Kim BS, Artis D. Group 2 innate lymphoid cells in health and disease. Cold Spring Harb Perspect Biol. 2015.
28. Rudmik L, Soler ZM, Mace JC, DeConde AS, Schlosser RJ, Smith TL. Using preoperative SNOT-22 score to

inform patient decision for Endoscopic sinus surgery. Laryngoscope. 2015 Jul;125(7):1517-22.
29. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B *et al*. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. Nat Immunol. 2011;12(11):1055–62.
30. Zhao A, Wang H, Wu H, Yang Y, Xu H, Wang D. Quantitative analysis of interleukin-5 mRNA and protein in nasal polyps. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2014; 28:1053-6.
31. Kubota K, Takeno S, Taruya T, Sasaki A, Ishino T, Hirakawa K. IL-5 and IL-6 are increased in the frontal recess of eosinophilic chronic rhinosinusitis patients. J Otolaryngol Head Neck Surg. 2017; 46:36.
32. Bachert C, Gevaert P, Holtappels G, Cuvelier C, Van Cauwenberge P. Nasal polyposis: from cytokines to growth. Am J Rhinol. 2000; 14:279- 90.
33. Allen JS, Eisma R, Leonard G, Kreutzer D. Interleukin-3, ınterleukin-5, and granulocyte-macrophage colony-stimulating factor expression in nasal polyps. Am J Otolaryngol. 1997; 18:239-46.
34. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the ınterleukin-10 receptor. Annu Rev Immunol. 2001; 19:683-765.
35. Adnan Ekinci, Muge Ozcan, journal of clinical and experimental investigations. 2018; 9; 2:72-75.