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Original Research Article

Bacterial Evaluation and Some Allergic Mediators of Chronic Rhinosinusitis with Nasal Polyps

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Abstract

Chronic rhinosinusitis with nasal polyps (CRSwNPs) is a disorder characterized by persistent eosinophilic Th2 inflammation with sinonasal microbial colonization. It has been postulated that a compromised local mucosal immune response and microbial by-product like super-antigen underlies disease pathogenesis
The affected sinuses fluid swabs and blood samples were collected from 50 a CRSwNP patients to isolate associated bacterial species and to measure serum IL-4 and total IgE and IgG levels. The same sample were collected from health subject as a control study. The sinonasal tract was colonized by *S.aureus H.influenzae S.pneumoniae P.aeruginosa Morexella.catarrhalis S.pygenes S.intermedius*, where the high prevalence rate was 34% of *S.aureus* , while the low prevalence rate was 2% of *S. intermedius*. ELISA revealed an elevation of serum IL-4, and total IgE and IgG levels in the CRSwNP patients compared with the control subject.
Bacterial colonization in CRSwNp is implicated with pathgenesis of this disease. The high levels of serum IL-4 and total IgE and IgG which were synthesized locally in the mucosa and diffuse to the blood , serve as an indicator of eosinophilic Th2- inflammation disorder.
of CRSwNP

Key words: Chronic rhinosinusitis with nasal polyps, bacterial colonization, Th2- immune response.

التقييم البكتيري وبعض وسائط الحساسية لالتهاب الأنف والجيوب الانفية المزمن مع الزوائد الانفية

الخلاصة

التهاب الانف والجيوب الانفية المزمن مع الزوائد الانفية هو اضطراب يتميز بسيادة الالتهاب الحمضي المتوسط بالخلايا التائية المساعدة النمط الثاني مع الاستيطان المايكروبي ويعتقد ان انخفاض الاستجابة المناعية الموضعية المخاطية مع النواتج المايكروبية مثل المستضد الفائق هو راء توليد هذا المرض.تم جمع عينات مسحات لسائل الجيوب الانفية المصابة وكذلك عينات الدم من خمسين شخص مصاب بالتهاب الانف والجيوب لغرض عزل الاجناس البكتيرية ذات العلاقة وكذلك لغرض قياس مستوى الانترليوكين٤ المصلي وكذلك قياس الكلوبيولين المناعي الكلي نوع اي والكلوبيولين المناعي الكلي جي. استعمرالمجرى الانفي الجيبي من قبل بكتريا المكورات العنقودية والمستدميات النزلية والمسبحيات الرئوية والزوائف الزنجارية والموركسيلا النزلي والمسبحيات القيحية والمسبحيات المتوسطة حيث كان اعلى نسبة انتشار هو ٣٤% لبكتريا المكورات العنقودية واقل نسبة انتشار هو ٢% لبكتريا المسبحيات المتوسطة . اظهرت نتائج الاليزا ارتفاع مستوى الانترليوكين ٤المصلي وكذلك ارتفاع مستوى الكلوبيولينات المناعية الكلية نوع اي وجي لمرضى التهاب الانف والجيوب الانفية مقارنة بالاشخاص الاسوياء.

يتورط الاستيطان البكتيري في توليد مرض التهاب الانف والجيوب الانفية المزمن مع الزوائد الانفية . ان ارتفاع مستوى الانترليوكين ٤ المصلي والكلوبيولين المناعي الكلي نوع اي وجي المتكونة موضعيا في الطبقة المخاطية يتم انتشارها الى الدم لتخدم كمؤشر على الالتهاب الحمضي المتوسط بالخلايا التائية المساعدة النمط الثاني لمرض الالتهاب الانف والجيوب الانفية المزمن مع الزوائد الانفية.

الكلمات المفتاحية: التهاب الانف والجيوب الانفية المزمن مع الزوائد الانفية، الاستيطان البكتيري، الاستجابة المناعية المتوسطة بالخلايا التائية النمط الثاني.

Introduction

Chronic rhinosinusitis (CRS) is a persistentinflammatorydisorder affected the nose and one or more of the paranasal sinuses mucosae [7]characterized by at least 8-12 weeks of at least 2 symptoms, like nasal obstruction and congestion, nasal discharge, facialpain or pressure and reduction or loss of smell [2,14]. The microbial present like bacteria and fungi represent as secondary infectious agent that contribute with persistence and severity of the disease,these microbes have advantage from the reduction of innate immune response resulted fromaltered epithelium, ciliary dysfunction, and mucus hyperplasia. CRS have been primarily divided intotwo groups: CRS with nasal polyps (CRSwNPs) and CRS withoutNPs(CRSsNP)[8,9].

In CRS wNP the tissue inflammatory processes are eosinophilia associated with TH_2- immune response that stimulates secretion of many interleukins and kemokines that together drive the influx eosinophils into the tissues causing IgE- mediated allergy as well as to the goblet cells metaplasia[16]. *In vitro* studies have suggested an important role for IL-4,IL-5 and IL-13 in allergic inflammation through the induction of immunoglobulin isotype switching to IgE in B cells.In contrast to the CRsNPthe inflammatory processes are neutrophila and associated with Th-1 polymerization and secretion of cytokines like gamma interferon and IL-1,IL-8 that induce cell-mediated immunity as phagocytosis[1,3].

The isolation of a microbes from CRS is difficult because the aspirated sample were performed after or during the antimicrobial treatment, although bacterial invasion by*Streptococcuspneumoniae, Haemophilusinfluenzae,* and *Moraxellacatarrhalis* as well as Staphylococcus aureus, Pseudomonas aeruginosa had been characterized in CRS diseases. The first three organisms can also found in acutesinusitis[9].

previous study demonstrated that the bacterial biofilmcreate in CRS as a result in alteration of innate immune response especially a reduction of the antimicrobial molecules expression of the sinonasalepithelium. Among bacteria that form biofilom are *S. aureus*,it secret superantigen like entertoxin, which provoke Th2-pathogenic processes and a elevation in IgE level which directed against *S.aureus*toxin[11].

Our studyhave two parameters: first,bacteriology which includes isolation and characterization of bacteria from sinuses fluid samples.

Second,immunology which includes evaluation of serum Il-4, total IgE and total IgG,we hypothesized that an elevation of seum IL-4,IgE may be indicated for Eosinophilic CRSwNP

Material and Methods

Sample collection

Three Swab samples of the inflamed sinuses fluid were collected by specialist physician during endoscopy sinus surgery from each of fifty adult CRS patients(mean age 21, male 28 female:22) Attending to Hilla teaching hospital, Department of Otolaryngology at a period from Dec 2013 to Feb 2014.Additionally to the blood samples which were collected in a gel tubes to obtain serum for serological examination. The Swabs of normal sinonasalmucous and blood samples were collected from 10 health subject representing the study control. The study group and control group had negative history of a drug allergy, asthma and immunodefficiency diseases.

Associated bacterial isolation

Associated bacterial species were isolated by incubation of each sample on blood agar chocolate agar and Mac Conkey agar at 37 C for 48 hour, pure culture was made of each isolate for microscopical (using classical Gram stain),cultural (shape,size, presence of blood haemolysis zone… etc) and biochemical identification (using conventional API of enterobacteriacae and gram positive bacteria (Biomereux, France)

Serological examination

the Serum was separated at 3000 r/ m to evaluate IL-4, and total IgE and IgG concentration levels by ELISA technique according to the instructions of the manufactured companies (CusabioBiotech Co.,LTD; InterMedical Diagnostics.it and Diagnostic Automation, INC.)respectively

Statistical analysis

All analyses were conducted using SPSS 18.0 The Student's t test(two-tailed, unequal variance) was chose to analyze the significance of differences betweenexperimental groups. Data with a P value of ≤0.05 was considered to be significant.

Results

Bacterial evaluation

According to the microbiological examination of the inflamed sinus swabs taken from the CRS patients in the present study revealed that a presence of various type of bacterial species(Table-1), where the high prevalence rate was 34% of *S.aureus*, while the low prevalence rate was 2% of *S. intermedius*. The sinonasal mucous swabs from the normal subject, seven out of ten samples only had positive culture of *S.epidermedis*.

Table1: Associated bacterial species with CRS with nasal polyps

Bacterial species	Occurrence	%
S.aureus	17	34
H.influenzae	13	26
S.pneumoniae	8	16
P.aeruginosa	7	14
Morexella. Catarrhalis	2	4
S.pygenes	2	4
S.intermedius	1	2
Total	50	100

Serological evaluation

Serum IL-4 level

The serum IL-4 concentration was elevated above 60.27 p/ml in a CRS patients , the mean was 122,192 p/ml compare with control subject the mean was 48,505 p/ml (Figure-1, A). The elevation of IL-4 level was statistically significant P≤0.05.

Total IgE level

The CRS patients showed significant elevation in total IgE concentration in their serum, the concentration was above 80.81 IU/ml compared with control subject which showed normal values of IgE. the means were 358.56 IU/ml and 55.35 IU/ml respectively (Figure-1,B). P≤0.05.

Total IgG level

The CRS with nasal polyps stimulates slight increase in the total serum IgG concentration,where the mean value of IgG in patient was 1.415 compare with those of normal subject was 1.343. (Figure-1, C) P≥0.05

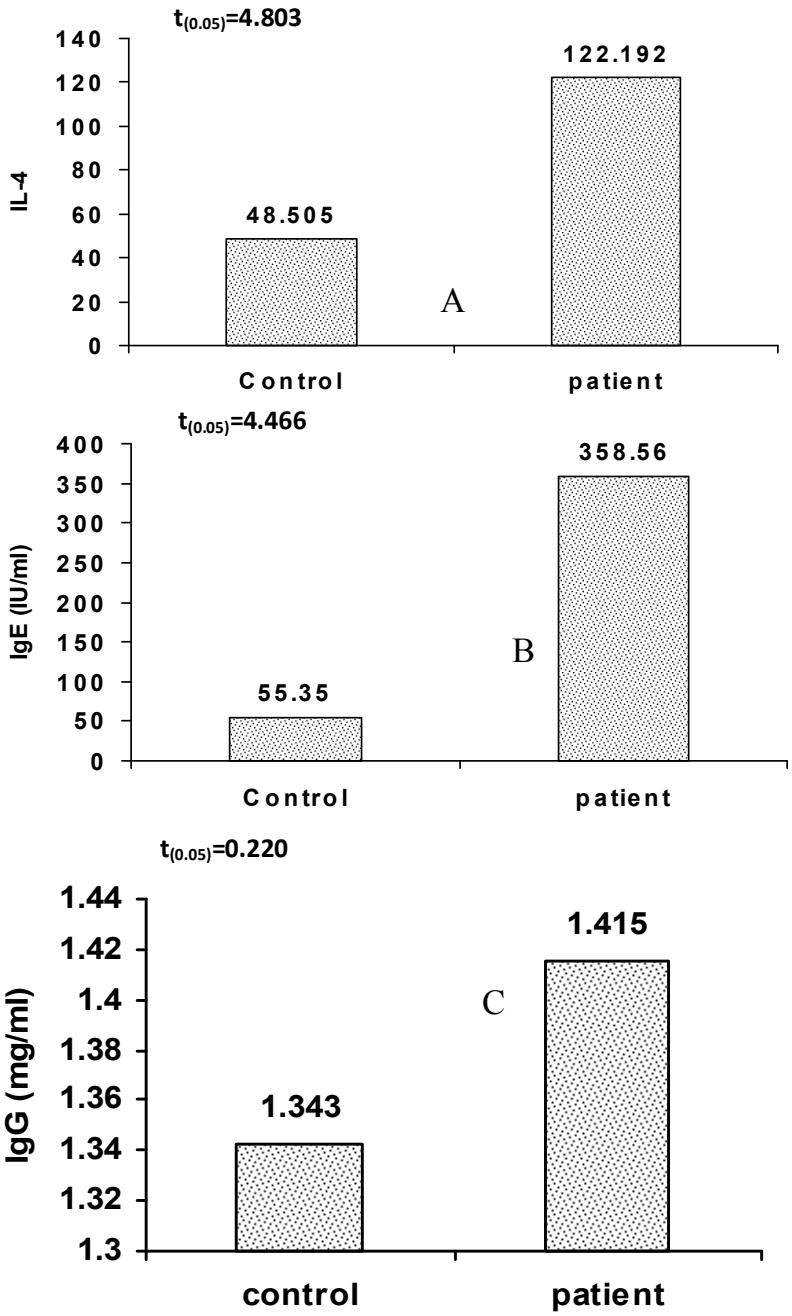

Figure1: Serum IL-4 , Total IgE and Total IgG concentration measured by ELISA . Results wereexpressed as a means ± standard error. Control = normal subject without CRS; patient: CRSwNP undergo subject

Discussion

Several studies have attempted to explain the pathophsiology of CRS. Some studies had adopted that CRSwNP appears to be an inflammatory disorder of the local mucosal immune system.Failure of epithelial cells to identify potential pathogens which resulted from decrease in expression of innate immune genes related to microbial recognition by sinonasal epithelial cells contribute to the development of microbial colonization in CRSwNP [8,13,17].

In present study, we recovered some types of bacterial species from affected sinuses, where the high isolation was is 34% of *S.aureus*.

An infectious etiology forCRS has long been suggested. Although some studies [18, 19] strongly implicatebacterial infection with CRS. An interesting study examined the microbiology of sinus aspirates during the transition fromacute rhinosinusitis to CRS [18]. Patients in the studyhad failed to respond to antibiotic treatment and had sequentialcultures performed over a 5- to 7-week periodafter the initial acute infection. This data provides evidence that bacteriology in CRS is different from that of control patients before acquisition of CRS. the flora of the paranasal sinuses seems to be altered, with a higher prevalence of Staphylococcus species and anaerobes. This flora is often polymicrobial and may exhibit significant antibiotic resistance

Initially, typical bacteria foracute rhinosinusitis were recovered, including *Streptococcuspneumoniae, H.influenzae,* and *M.catarrhalis*.These organisms can also be found in chronicsinusitis, as well as *S. aureus, P.aeruginosa*, and certain anaerobes [4,6,9].

The elevated levels of the serum IL-4 IgE and IgG in CRS patients it leads us to propose that these humoral molecules were produced locally and, rather than accumulating in nasal secretions diffuse into the blood to elevate serum levels.

In vitrostudies have suggested an important role forIL-4 and IL-13 in allergic inflammation through the inductionof immunoglobulin isotype switching to IgE in B cells [3, 20]. The possibility of local IgE production in chronic rhinosinusitis has been conducted by previous studies[1,3,10].

Ramanathan et al [13] had been shown that IL-4acts on SNECs in vitro to down-regulate production of TLR9(TLR9, in which its ligand is bacteria)

In CRSw NP, there is an increase in the Th2 cytokineslike IL-4, IL-5 and IL-13 and the intensity ofeosinophils in the tissues of these patients is markedlyincreased in the presence of co-existing asthma or positiveallergy skin tests. The increased presence of IL-4 andIL-13 can play a role in upregulating VCAM-1 and thusfacilitates the further infiltration by eosinophils[2].

Recent studies have supported a superantigenhypothesis, suggesting that toxins secreted by S aureus, in somecases protected by biofilms or sequestered within epithelial cells,mediate inflammation in patients with CRSwNP through direct stimulation of Th-2 cells, leading to cytokine release and local polyclonalIgE responses [2,12].

References

1- Zele, T.V., Claeys , A., Gevaert, P., Maele, G.V., Holtappels, G., Cauwenberge, P.V and Bachert, C. (2006) Differentiation of chronic sinus diseases by measurement of inflammatory mediators. Allergy. 61: 1280–1289.

2- Bachert,C., Pawankar,R., Zhang3,L,. Bunnag, C., Fokkens, W.J., Hamilos, D.L., Jirapongsananuruk, O., Kern, R., Meltzer8,E., Mullol9,J., Naclerio, R., Pilan11,R., Rhee12,C.S., Suzaki13,H., Voegels14,R and Blaiss, M. (2014). ICON: chronic rhinosinusitis. World Allergy Organization Journal, 7:25

3- Ghaffar, O., Durham S.R., Al-Ghamdi, K., Wright, E.,Small,P., Frenkiel, S., Gould, H. J. and Hamid, Q.(1998). Expression of IgE Heavy Chain Transcripts in the Sinus Mucosa of Atopicand Nonatopic Patients with Chronic Sinusitis. Am. J. Respir. Cell Mol. Biol. Vol. 18, pp. 706–711

4- Araujo, E., Dall, C., Cantarelli, V., Pereira, A.,Mariante, A.R.(2007).Microbiology of Middle Meatus in Chronic Rhinosinusitis. Brazilian J. Otorhinolaryngology. 73(4):549-55

5- Yoshida, S.H and Gershwin, M.E. (1998). Immunity, infection, and nasal disease.Clin Rev Allergy Immunol., Volume 16, Issue 1-2, pp 3-24.

6- Wood, A.J., Fraser, J.D., Swift, S., Patterson- Emanuelson, E.A.C., Amirapu, S and Douglas, R.(2012). Intramucosal bacterial microcolonies exist in chronic rhinosinusitis without inducing a local immune response. Am. J Rhinology and Allergy 26(4):265-70.

7- Helms, S and Miller, A.L.(2006).Natural treatment of Chronic Rhinosinusitis.Alternative medicine review. 11(3) :196-207

8- Leung, R. S, andKatial, R. (2008).The Diagnosis and Managementof Acute and Chronic Sinusitis. Elsevier. Prim Care Clin Office Pract .35 : 11–24

9- Slavin, R. G.,. Spector, S. L and Bernstein, L.(2005). The diagnosis and management of sinusitis:A practice parameter update. J.Allergy. Clin. Immunol.116:S13-47

10- Baroody, F.M, Suh S.H, and Naclerio R.M. (1997). Total IgE serum levels correlate with sinus mucosal thickness on computerized tomography scans.J.Allergy.Clin.Immunol. 100(4):563-8

11- Kennedy, J. L and Borish Larry. (2013) Chronic rhinosinusitis and antibiotics: The good, the bad, and the ugly. Am. J. Rhino.l Allergy. 27, 467–472

12- Tieu, D. D.,. Kern, R. C and. Schleimer, Robert. P. (2009). Alterations in epithelial barrier function and host defenseresponses in chronic rhinosinusitis. J.Allergy.Clin.Immunol. 124(1):37-24.

13- Ramanathan, M., Lee, W.N.,Spannhake,E.W ,and Lane, A.P. (2008). Th2 cytokines associated with chronic rhinosinusitis with polypsdown-regulate the antimicrobial immune function of humansinonasal epithelial cells.Am J Rhinol. 2008; 22(2): 115–121.

14- Antunes, M.B., Gudis, D.A., Cohen, N.A. (2009). Epithelium, cilia, and mucus: their importance in chronic rhinosinusitis. Immunol Allergy Clin North Am . 29(4):631–643.

15- Ramanathan M Jr, Lane AP. (2007). Innate immunity of the sinonasal cavity and its role in chronic rhinosinusitis. Otolaryngol Head Neck Surg;136:348–356

16- Van Bruaene, N., Perez Novo, C., Deruyck, N., Holtappels, G., Van Cauwenberge, P., Bachert, C. (2012). Inflammation and remodeling patterns in early-stage chronic rhinosinusitis. ClinExp Allergy 42:883–890.

17- Van Zele T, Gevaert P, Watelet JB. (2004). Staphylococcus aureus colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. J Allergy ClinImmunol;114:981–3.

18- Brook I, Yocum P, Frazier EH (1996). Bacteriology and beta-lactamase activity in acute and chronic maxillary sinusitis. Arch Otolaryngol Head Neck Surg. 122:418–423.

19- Bhattacharyya N, Kepnes LJ. (1999). The microbiology of recurrent rhinosinusitis after endoscopic sinus surgery. Arch Otolaryngol Head Neck Surg,125:1117–1120

20- Coffman, R. L., D. A. Lebman, and P. Rothman. (1993). Mechanism and regulation of immunoglobulin isotype switching. Adv. Immunol. 54:229–270