



Expression of Eotaxin and RANTES in Nasal Polyps versus Healthy Nasal Mucosa, a Case Control Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors YN, GB and OA designed the study, wrote the protocol, carried out the processing of the samples, wrote the first draft of the manuscript, managed the literature searches and performed analyses of the study results. Author AES was responsible for obtaining samples during surgical interference for studied cases and control. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/26409

Editor(s):

(1) Patorn Piroomchai, Department of Otorhinolarngology, Khon Kaen University, Thailand.

Reviewers:

(1) Dario Buccheri, University of Palermo, Italy.

(2) Rogerio Pezato, Federal University of São Paulo, Brazil.

(3) Anonymous, University of Virginia, USA.

(4) Karol Zelenik, University Hospital Ostrava, Czech Republic.

Complete Peer review History: <http://sciencedomain.org/review-history/14824>

Original Research Article

Received 15th April 2016

Accepted 9th May 2016

Published 30th May 2016

ABSTRACT

Aims: To investigate the levels of the chemokines; eotaxin and RANTES in chronic rhinosinusitis with nasal polyposis in comparison to their levels in control healthy nasal mucosa to evaluate if they might play a role in pathogenesis.

Study Design: We performed a prospective case control study.

Place of Study and Duration: This study was performed in Otorhinolarngology Department Mansoura University Hospitals, Egypt.

Methodology: It included 60 patients suffering from chronic rhinosinsuitis with nasal polyposis, in addition to 20 subjects that were included as control. Nasal tissue samples were collected from all cases and control to estimate the levels of eotaxin and RANTES by ELISA.

Results: The estimated levels of eotaxin and RANTES in nasal polyps were higher than their measured levels in healthy nasal mucosa from control group and the results were

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statistically significant.

Conclusion: The measured levels of eotaxin and RANTES suggest that they may have a role in pathogenesis of chronic rhinosinusitis with nasal polyposis and can be considered as a good target for medical therapy until supported by further studies.

Keywords: Nasal polyposis; eotaxin; RANTES; E-selectin; enzyme linked immunosorbent assay.

ABBREVIATIONS

NPs; Nasal polyps, RANTES; Regulated on Activation Normal T Expressed and Secreted, CT; computed tomography, ELISA; Enzyme linked immunosorbent assay, OD; Optical density.

1. INTRODUCTION

Nasal polyposis is a chronic inflammatory disease affecting upper airways. It represents edematous inflammatory benign lesion affecting approximately 1–4% of the general population, especially attacking elderly males [1]. Its exact etiopathogenesis is poorly understood leading to poor impact on the therapeutic intervention and so its frequent recurrences. The genetic predisposition besides external precipitating factors such as allergy, bacterial infections (precisely in the presence of biofilms and/or superantigens), fungal infections, cystic fibrosis, aspirin intolerance and asthma may play a role in development of chronic rhinosinusitis with nasal polyposis [2-5]. Besides, the extraesophageal reflux that can occur simultaneously with chronic rhinosinusitis and bronchial asthma predisposing to nasal polyps (NPs) [6].

Nasal polyps (NPs) are semi translucent, fine, gelatinous and pale out growths that originated from the mucous membrane of the ostiomeatal complex. They always arise from nasal or paranasal sinus mucosa and then prolapse into the nasal cavity [7]. This probably occurs due to the release of proinflammatory cytokines from epithelial cells as a result of contact between two surfaces of mucosa at this narrow region [8].

The typical histological appearance of nasal polyps is a heavy inflammatory infiltrate, loose fibrous connective tissue with tissue edema, and a thickened basement membrane [9]. Multiple cell types especially epithelial cells, eosinophils, fibroblasts, lymphocytes and mast cells were discovered to be involved in the inflammatory reaction in nasal polyps [10].

The chemokines RANTES (Regulated on Activation Normal T Expressed and Secreted), IL5 and eotaxin are considered essential recruiters and activators of both eosinophils, basophils, besides Th2 lymphocytes and mast cells. So they have been hypothesized to be

responsible for eosinophilia in the tissue stroma of the polyp [11].

The interaction of these chemokines with their receptors, CCR-5 and CCR-3 is essential in chronic rhinosinusitis with nasal polyposis [12]. These receptors are found in the normal nasal mucosa and they have been widely studied in allergy, where clear tissue eosinophilia has also been observed. They are expressed on eosinophils and in basophils as well [13]. Tumor necrosis factor alpha (TNF-alpha) and interleukin-1 beta (IL-1 beta) induce gene expression and protein synthesis of these chemokines in nasal polyp fibroblasts but with time and dose-dependency [14].

The recruitment and activation of leukocytes into the nasal polyps requires the expression of adhesion molecules by the endothelium to promote this recruitment in a multistep process involving leukocyte adhesion followed by rolling on the surface of activated endothelial cells, then transendothelial migration [15]. Adhesion molecules involved in this process include L-selectin, P-selectin, and E-selectin, in addition to intercellular adhesion molecule 1 (ICAM1) and vascular cell-adhesion molecule 1 (VCAM1) [16]. As the pathogenesis is mainly based on inflammatory process so using different antiinflammatory agents can modulate it [17].

The objective of the our study was to investigate the levels of the chemokines eotaxin and RANTES in chronic rhinosinusitis with nasal polyposis in comparison to their levels in control healthy nasal mucosa to evaluate if they might play a role in pathogenesis.

2. MATERIALS AND METHODS

2.1 Patient Selection

A total of 60 patients who met the inclusion criteria and admitted into the Otorhinolaryngology

Department at Mansoura University Hospital were included in this study. It was carried out over the period from November 2014 to May 2015. The protocol of the study was reviewed and approved by our institutional review board. A written and informed consent was obtained from all patients. They were 21 females and 39 males. Their ages ranged from 19 years to 65 years old.

The inclusion criteria included; diagnosis of allergic rhinitis which was confirmed by clinical history, more than 18 years old, clinical examination, having a positive skin test for a certain inhalant allergen as pollen or dust mite, and confirmed by CT scan showing evidence of the polyp soft tissue mass. All studied cases were non smokers.

Twenty subjects were included as control group who were non smokers, having no positive skin-prick in response to common aeroallergens or history of any respiratory disease and free CT scan, mostly admitted for rhinoplasty. They were 8 females and 12 males. With age ranged from 25 years to 62 years old. Nasal tissue from the control group was taken from the inferior turbinate tissue during the septoplasty operations.

Surgically removed nasal polyps from cases and nasal tissue from control group were put in sterile saline and minced using a homogenizer. The produced fluid was then centrifuged at 5000 rpm for 5 minutes then the supernatant was stored at -30°C until further processing.

2.2 Estimation of Eotaxin Levels

Using Human Eotaxin ELISA Kit (BioSource, Nivelles, Belgium) and according the manufacturer's rules, 50 µl of the standard diluent buffer were added to the wells reserved for samples and zero well. Fifty microns of standards, samples or controls were added to the appropriate microtiter wells then 100 µl of biotinylated anti-Eotaxin (Biotin Conjugate) solution were pipetted into each well. After incubation for 2 hours, the wells were aspirated and washed. One hundred µl Streptavidin-HRP working solution were added to each well and incubated for 30 minutes followed by thorough aspiration and washing. One hundred µl of TMP color developing agent were then added to each well. After incubation for 30 minutes, 100 µl of stop solution were added to each well. The wells were read at 450 nm absorbance (using ELISA

reader Spectra III, Austria) having blanked the plate reader against a TMP color developing agent blank composed of 100 µl each of TMP color developing agent and stop solution. Its sensitivity was < 2 pg/ml.

2.3 Estimation of RANTES and E-selectin Levels

They were estimated as previously described for eotaxin levels measurements according to the manufacturer instructions using human Rantes ELISA Kit (BioSource, Nivelles, Belgium) and human E-Selectin ELISA Kit (RayBiotech, Georgia, USA) respectively. Their sensitivities were < 3 pg/ml and 30 pg/ml respectively.

2.4 Statistical Analysis

The data were presented in the form of numbers, percentages, means with standard deviation and medians. Data were analyzed using the statistical package for social science (SPSS v17) program under windows using chi-square and Mann-Whitney Test. Results were considered significant when P value < 0.05.

3. RESULTS

In our study sixty nasal polyp tissue samples were taken from 60 patients and 20 inferior turbinate tissue samples as a control group. The age of studied cases ranged from 19 years to 65 years (mean 46.5±16 years).

The measured levels of eotaxin in nasal polyps surgically removed from patients suffering from chronic rhinosinusitis with nasal polyposis were higher than the measured levels in healthy nasal mucosa from control group and the results were statistically significant. The detected RANTES levels were also significantly higher than those detected in control group. The median of detected levels of eotaxin and RANTES in polyposis patient was 98.58 pg/ml and 127.51 pg/ml respectively whereas the control group had a median of 1.41 pg/ml and 1.80 pg/ml respectively Table 1.

On the contrary E-selectin measured values showed no reliable differences between nasal polyposis patients and healthy control ones. The median was found to be 95.01 pg/ml in nasal polyposis patients versus 92.00 pg/ml in control group Table 1.

Table 1. Results of measuring eotaxin, RANTES and E-selectin in nasal tissue of patients with nasal polyposis and control group

	Eotaxin (pg/ml)		RANTES (pg/ml)		E- selectin (pg/ml)	
	(Min-Max)	Median	(Min-Max)	Median	(Min-Max)	Median
Cases	86.00-375.10	98.58	60.00-356.00	127.51	87.00-156.00	95.01
Control	1.01-3.01	1.41	0.60-3.00	1.80	81.00-145.00	92.00
P value	<0.0001*		<0.0001*		<0.094	

* means significant

4. DISCUSSION

Cytokines, chemokines, adhesion molecules, and metalloproteinases are considered main targets for a better understanding and evaluation of the mechanism of the inflammatory cascade in chronic rhinosinusitis with NPs and so the determination of therapeutic targets for the inhibition of such inflammatory mediators [13,18-20].

Our mentioned results regarding eotaxin and RANTES were in accordance with what estimated by Bartels et al. [21] as they recorded increased expression of eotaxin and RANTES mRNA in nasal polyps when compared to normal nasal mucosa of control ones and this indicated that these chemokines are produced and mainly act at a local level. Also in congruency with our work at the nasal tissue, Marcella et al. [22] hypothesized that RANTES is locally produced within the nasal polyp microenvironment and is responsible for the inflammatory cell recruitment present in nasal polyposis. These results clearly showed that RANTES expression and production increase in nasal mucosa of patients with nasal polyposis compared to the same mucosa in healthy patients.

These findings also run in parallel with Olze et al. [23] who noted a higher concentration of proteins of eotaxins in eosinophilic polyps than in normal nasal concha mucosa using ELISA technique for estimation. Our results similarly agrees with Chao et al. [24] who said that eotaxin levels were elevated in chronic rhinosinusitis and even these levels were positively correlated to disease severity.

Our findings about measured levels of E-selectin follows what estimated by Ural et al. [25] as they discovered that E-selectin levels were similar in all studied groups of nasal polyposis and control. But it was in contrast to what Kang et al. [26] announced in their study as they estimated that the expression of E-selectin in the vascular endothelium and the matrix of nasal polyps were

significantly higher than those of inferior turbinates of healthy mucosa. They depended on immunohistochemistry to detect the presence and the levels of E-selectin which may be more sensitive and better than ELISA in E-selectin detection. These contradictory results need more studies and investigations over a larger group of patients for assurance of results.

A limitation to our study was obtaining control samples from the inferior turbinate nasal mucosa tissue instead of the middle turbinate of which we are aware that they have different mechanical properties [27], but represent more ease and feasibility for obtaining samples but in turn provide insights for better obtaining the samples from the middle turbinate in future studies through providing more facilities for such maneuver.

5. CONCLUSION

We concluded that eotaxin and RANTES are suggested to have a role in the pathogenesis of the chronic rhinosinusitis with nasal polyposis and so can be considered as a good target for medical therapy until supported and approved by further studies.

ETHICAL APPROVAL

All authors declare that the study protocol has been examined and approved by the institutional review board of Mansoura faculty of medicine and has therefore been performed in accordance with the ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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