Inflammatory mediators, cell counts in nasal lavage and computed tomography of the paranasal sinuses in atopic children

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Abstract

Objective: the aims of this study were to evaluate inflammatory cells, the profile of inflammatory mediators in nasal lavage (NL), and the involvement of the paranasal mucosa in atopic infants with no symptoms of sinusitis.

Methods: 48 atopic patients with allergic rhinitis (AR), and 33/48 patients with asthma were studied; the control group consisted of 13 nonatopic children. Those individuals with acute, chronic or recurrent sinusitis were excluded. The involvement of the paranasal mucosa was assessed by coronal computed tomography (CT) and graded by a standard protocol (0-30). A CT score greater than or equal to 12 indicated extensive involvement. Nasal lavage was used to quantify total and differential nasal cell counts. An aliquot of the supernatant was used for determining inflammatory mediators: interleukin-8 (IL-8), myeloperoxidase (MPO), and eosinophil cationic protein (ECP). Albumin was used as a marker for increased vascular permeability. These measurements were performed on all of the atopic patients and in 6/13 patients in the control group. The three groups were submitted to spirometry and complete blood cell count.

Results: extensive involvement of the paranasal mucosa was observed in 7/33 (21%) of asthmatic patients (Group I) and 2/15 (13%) of those with allergic rhinitis (Group II). The highest CT score in the control group (Group III) was 7. Total cell and eosinophil count/ml and albumin concentration in nasal fluid were higher in asthmatic patients whose CT score was greater than 12. Interleukin-8 concentration, number of neutrophils and epithelial cells/ml in nasal fluid were similar in the three groups. A positive correlation between CT score, peripheral blood eosinophilia, number of eosinophils/ml and eosinophil cationic protein concentration was found in the nasal fluid of atopic children (n=48). There was an association between number of neutrophils and titers of interleukin-8 and myeloperoxidase, and between interleukin-8 and eosinophil count.

Conclusions: in asthmatic patients with no symptoms of sinusitis, the extensive involvement of the paranasal mucosa is associated with blood and nasal lavage eosinophilia and cellular activation. Neutrophil infiltration and activation were not related to increased involvement of the paranasal mucosa.


Introduction

Sinusitis is defined as the inflammatory process of one or more paranasal sinuses that is frequently associated with allergic rhinitis and asthma.1 The literature does not present a study on the incidence of bacterial sinusitis in allergic and nonallergic patients, nor on whether the earlier present more acute or severe disease than the latter.2

Sinusitis can coexist with, or be the cause of asthma. The exact mechanism responsible for this association, however, is still not well-understood. Moreover, the lack of uniform procedures for the diagnosis of sinusitis has allowed for conflicting results.3 Allergic children with chronic respiratory symptoms have presented increased radiological alterations of the facial sinuses, from mucosal thickening to

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Patients and Methods

We consecutively selected 48 atopic outpatients being followed up at the Pediatric Allergy and Immunology Outpatient Clinic of the Hospital das Clínicas, teaching hospital of the Universidade Federal do Paraná. All 48 patients had allergic rhinitis, and 33 out of the 48 patients had asthma associated with allergic rhinitis (all positive allergy skin test responses to the acarus D. pteronyssinus).

The control group included 13 nonatopic children followed up at the Neuropediatrics Service. These patients were submitted to cranial CT scan for investigation for epilepsy. This group was examined using anamnesis and physical examination for symptoms and signs of allergic rhinitis and/or asthma; moreover, these patients were selected according to negative skin test response in order to exclude atopy. We selected children with minimal age of 8 years and who cooperated with exams of pulmonary function, and CT scan of facial sinuses and of nasal washings.

The 61 patients were stratified according to diagnosis and to CT score. Group I included patients with asthma and allergic rhinitis (n=33); group II, patients with allergic rhinitis (n=15); and group III, patients who were nonatopic (n=13).

We excluded patients with history of recurrent or chronic sinusitis indicating symptoms of odynophagia, purulent nasal and postnasal secretion, nasal congestion, and productive night cough(4); with infection of upper and lower airways, respectively at 2 and 4 weeks prior to CT scan and spirometry; with presence of mechanical obstruction to rhinoscopy; with use of topic or systemic corticoids, respectively at 1 and 2 months prior to the study; and with use of antihistaminics on the week prior to the study.

We collected blood for hemogram examinations. Patients with 350 cells/mm³ or more were considered cases of eosinophilia. 12 Spirometry was carried out with electronic spirometer (Spirosift 3000; Fukuda Denshii, Japan). The forced expiratory volume in one second (FEV1) was expressed as percent of predicted. 13

During the examinations, patients presented symptoms of asthma and allergic rhinitis characterized, respectively, by recurrence of dry cough, wheezing, and/or dyspnea and by itching, sneezing, coryza, and/or nasal obstruction. 14 Cases of rhinitis were not treated. Asthma was treated with routine use of teophylline (5 mg/kg doses; three times a day) and of bronchodilator on demand.

We carried out coronal CT scans of the facial sinuses starting at the frontal sinus to the sphenoidal sinus (including the nasal cavity). Exams were assessed by a radiologist who was not aware of clinical status of patients. A standardized score was employed to assess the severity of involvement of the mucosa of paranasal sinuses. The areas assessed were divided into nasal cavity, osteomeatal complex, and paranasal sinuses. On the paranasal sinuses, each sinus was individually examined according to maximum thickness of the mucosa (mm); a score of 0 to 3 was used according to thickening of the mucosa. Quantitation of osteomeatal complex and nasal passage was scored 0 for absence of lesion, 1 for minimal involvement, 2 for moderate involvement, and 3 for severe involvement of the mucosa. The maximum score was 30 (21 points for facial sinuses, 6 for osteomeatal complex, and 3 for nasal passage). CT score

Coronal computed tomography is currently the optimal study for examination of paranasal sinuses. This exam allows for delineation of the osteomeatal unit and of the ethmoidal sinuses, which are important in the pathophysiology of sinusitis. CT scans also allow for determining extension of the involvement of the paranasal mucosa. 7

The histology of the nasal mucosa is similar to that of lower airways. The nasal mucosa can be easily accessed, thus allowing for study of the allergic process, especially in children. 8,9

Quantitative cytology of nasal secretions (QCNS) can help the investigation for rhinitis, but it is a complex, expensive, and slow procedure(10). QCNS has been simplified and assessed for precision and accuracy, in addition to being compared to differential count in nasal scrape. 11

On the one hand, due to reports of sinusitis deteriorating or causing asthma, the use of antibiotics is common in allergic patients with chronic respiratory symptoms. On the other hand, studies have increasingly shown the presence of radiological alterations of the facial sinuses in asthmatic and/or allergic rhinitis patients, with or without infectious sinusitis. These findings allow for questioning of the prolonged use of antibiotics in these patients, considering that others have reported improvement without the use of antibiotic treatment. 2

Our study was carried out with children with persistent asthma and/or perennial allergic rhinitis and asymptomatic for infectious sinusitis. Our objective was to assess the relation of cellularity and inflammatory mediators in nasal washings with the severity of tomography alterations of the paranasal mucosa of these patients.
sum was used to group patients according to severity of lesion to the paranasal mucosa. Scores from 0 to 11 indicated limited disease and scores greater than or equal to 12 indicated severe disease.15,16

Nasal washings were obtained according to the technique described by Bascom et al.,10 which we adapted for children. For the collection of nasal fluid, patient’s heads were in extended prone position at approximately 30 degrees. After occlusion of the rhynopharinx with the soft palate, 5.5 ml of saline solution were instilled in fractional doses into each nostril. After 10 seconds, patients flexed their neck and the material was collected into a graded, conical tube; the tube was kept in ice during the collection procedure. The volume recovered was registered immediately.10,17

Homogenization of nasal fluid was carried out with vigorous shaking of the material and centrifuging at 4 degrees C (1,000 g/5 min).10 Aliquots of the supernatant material were stored at -80 degrees C for determination of inflammatory mediators and cytokines. The sediment obtained was suspended in 10-ml phosphate buffer saline solution (PBS), shaken and centrifuged at 4 degrees C (1,000 g/15 min). We measured the final volume of the second sediment. A 10-µl aliquot was used to fill the Neubauer hemocytometer counting chamber to count the total number of cells per ml (100x and 400x magnification microscope). If the sample presented a high concentration, the preparation was diluted once more for total cell count using the formula below:

Total number of cells/ml = [(cell count) / (squares count)] ÷ [counting chamber conversion factor] x [dilution factor].

The Neubauer hemocytometer conversion factor was 10,000. The dilution factor used was the ratio final:initial volume of the sediment.18

After dilution of the sediment in PBS, cell concentration was adjusted to 500 cells/µl. Out of this sample, a 200-µl aliquot was centrifuged (LABHO CT-12 Cytocentrifuge) at 600 rpm for 6 minutes for the slides. The slides were stained using May-Grunwald-Giemsa stain for differential count of 100 cells in a 400x or 1,000x magnification microscope. Slides were examined by a pathologist who had no knowledge of clinical status of patients. The pathologist carried out the examination together with one of the authors. The total count per ml of each cell type was calculated multiplying total cell count per ml by the fraction obtained in differential diagnosis.10,11,19

Competitive radioimmunoassay using iodine 125-labeled albumin (Diagnostic Products Corporation; Los Angeles, CA) was used for detecting and quantitating albumin concentration in nasal fluid. The concentration of myeloperoxidase (MPO) in nasal fluids was measured by radioimmunoassay (Pharmacia & Upjohn Diagnosis - Uppsala, Sweden). The concentration of Eosinophil cationic protein (ECP) was measured by fluorometric enzyme immunoassay (FEIA; Pharmacia CAP System; Uppsala, Sweden). Finally, the concentration of interleukin-8 was measured by double-antibody enzyme immunoassay (R&D Systems, Minneapolis, MN).

We employed analysis of variance (ANOVA) for assessing differences in and between groups. Mann-Whitney test was used for comparison of total and differential cell counts of mediators and cytokines in nasal washings with nonpaired groups. Correlation was assessed using Spearman’s correlation coefficient. Fisher’s test with Yates’ correction was used to assess the differences between proportions. Significance level was considered at 5%. The statistical software employed was the STATISTICA (version 4.2).

Our study was approved by the Ethics Committee of the Hospital DAs Clínicas, Teaching Hospital of the Universidade Federal do Paraná. Informed consent was obtained from the parents or guardians of all patients in the study population.

Results

The 61 patients in our population were grouped according to diagnosis and CT scores. Group I included patients with asthma and allergic rhinitis (n=33); group II, patients with allergic rhinitis (n=15); and group III, patients who were nonatopic (n=13). The age averages and ranges of patients in groups I, II, and III were, respectively, 11.6 (9-15); 11.7 (8.8-14.5); and 11.8 (8-14) years. The female-to-male sex ratio of each group was of, respectively, 11:22; 5:10; 3:10.

Lower and upper limits of standard CT scores of facial sinuses were 0 and 30. Severe paranasal disease (CT score greater than or equal to 12) was diagnosed in 7 (21%) of patients in group I; and 2 (13%) in group II. All patients in group III presented scores less than 12 (Figure 1). The findings of anatomical variations were not associated with severity of lesions indicated by CT scores of facial sinuses.

Atopic patients presented eosinophilia, which was significantly more prevalent between asthmatic patients. We observed a positive correlation (rs=0.41; P=0.004) between peripheral eosinophilia and severity of mucosal involvement indicated by CT scores (Figure 2).

In group I, the distribution of patients according to severity of asthma in the categories of mild or moderate persistent was, respectively, of 24 and 9 cases; no patient presented severe asthma. The upper and lower limits of FEV1 for group I were 61 and 107%. The average FEV1 was smaller in the group with asthma and allergic rhinitis compared to controls. FEV1 was not different between asthmatics with CT score greater or less than 12. FEV1 was similar in the allergic rhinitis and control groups.
Nasal washing was carried out in all patients and the recovered nasal fluid was analyzed for total and differential cell count per ml. The volume percentage of nasal fluid recovered in all three groups was similar. We carried out analysis of inflammatory mediators on the nasal fluids of all atopic patients and in 6 out of 13 control patients.

The total cell count recovered from the nasal fluid of patients with asthma and allergic rhinitis was significantly greater than that of controls. There were no differences between the allergic rhinitis group and controls (Table 2). Total cellularity was greater in the subgroup of asthmatic patients with severe lesion indicated by CT scan (median=1,322 cells/ml).

Atopic patients presented prevalence of eosinophils in nasal fluid and a positive correlation between CT score and eosinophil count per ml (Figure 2). There was no correlation of CT scores of facial sinuses with other cells of the differential count, nor with total cellularity per ml of nasal fluid. The total neutrophils count per ml was similar in all three groups. The total epithelial cells count per ml was greater in the asthma and allergic rhinitis group in comparison to the allergic rhinitis group. The allergic rhinitis and control groups were similar as to the total epithelial cells count per ml (Table 2).

The total eosinophils count per ml was significantly greater in the group of asthmatics with severe involvement of the mucosa (median=912 eosinophils per ml) in comparison to the other groups, including that of asthmatics with facial sinuses CT scores less than 12 (median=309 eosinophils per ml). The total neutrophil and epithelial cell counts in nasal fluid were similar in all subgroups, independently of the severity of involvement of paranasal mucosa indicated by CT of facial sinuses.

Early analysis of inflammatory mediators and cytokines indicated differences only in level of MPO, which was greater in the control group in comparison to asthmatics. Assessment of groups according to CT scores indicated that this difference remained only in the comparison of controls and asthmatics with CT score less than 12. The level of albumin in nasal fluid was higher in asthmatics with CT scores of facial sinuses greater than 12 (median=85) in comparison to controls (median=45). We noted a wide variation in concentration of ECP in nasal fluid of our population. ECP was significantly greater in the group of asthmatics with CT scores of facial sinuses greater than 12.
The levels of interleukin-8 in nasal fluid were similar in all three groups despite the severity of involvement of paranasal mucosa according to CT of facial sinuses.

CT scores of facial sinuses were associated with ECP (Figure 2). Analysis of differential cell count indicated that only the concentration of eosinophils per ml presented a direct relation with the determinations of albumin (RS=0.49; P less than or equal to 0.001) and ECP (RS=0.55; P less than or equal to 0.0001).

Discussion

Rhinosinusitis are among the most frequent chronic diseases; they are commonly associated with allergic rhinitis, and high morbidity rates and treatment costs.1

Alterations in radiological findings of the facial sinuses are common in children with atopic asthma and associated allergic rhinitis. These findings are parallel to persistent symptoms of upper airways that simulate chronic sinusitis even in the absence of associated infections.

Pelikan and Pelikan-Filipek20 observed an increase in mucosal edema and/or opacification in 32 out of 73 patients through radiological examination of facial sinuses before and repeatedly after allergen challenge. These authors showed the relationship between sinusitis and allergic rhinitis.20

In our study, two out of 15 patients (13%) with perennial allergic rhinitis presented severe involvement of the mucosa at CT scan of facial sinuses. Asthma and rhinitis (group I) and allergic rhinitis (group II) patients presented active rhinitis; thus, the difference between these patients was presence or not of asthma. Since group I patients presented a more severe involvement of paranasal mucosa, we believe that the variable asthma, independently of its severity, is related to greater involvement of the paranasal mucosa. This could be the indication of a more severe inflammatory response of the respiratory mucosa.

The estimated incidence of sinusitis in asthmatic patients is of 40% to 75%. Despite the fact that others have indicated that sinusitis precipitates or deteriorates asthma, the question of whether they simply coexist or are the target of the same inflammatory process in different parts of the respiratory tract still remains unanswered.1

Pfister et al. observed that computed tomography showed at least minimal mucosal thickening in any of the paranasal sinuses in 74% of the patients. Acute sinusitis was not common.5

Paranasal mucosal thickening was also reportedly frequent in the investigation of 100 asthmatic patients who presented persistent upper airway symptoms, and were
Table 3 - Inflammatory mediators in nasal fluid of patients of the three groups

<table>
<thead>
<tr>
<th>Grupo</th>
<th>n</th>
<th>Albumina (mcg/l)</th>
<th>ECP (mcg/l)</th>
<th>MPO (mcg/l)*</th>
<th>IL-8 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Asthma and AR)</td>
<td>33</td>
<td>74 (10–205)</td>
<td>55 (2–3,614)</td>
<td>246 (12–612)</td>
<td>831 (66–2,245)</td>
</tr>
<tr>
<td>II (AR)</td>
<td>15</td>
<td>57 (6.6–402)</td>
<td>41 (0.02–266)</td>
<td>112 (12–1,368)</td>
<td>692 (103–4,000)</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>45 (12–98)</td>
<td>34 (5–124)</td>
<td>427 (195–846)</td>
<td>1,243 (513–2,488)</td>
</tr>
</tbody>
</table>

‡ Median (Limits)
* MPO: Group I x Group III, P=0.03

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submitted to radiological examination of the facial sinuses. Thickening greater than 6 mm was associated with productive cough and bacterial sinusitis; this was not observed for patients with thickening less than 2 mm. The authors concluded that this is an indication of allergic sinus disease.21

Zimmerman et al. carried out study on the relation of radiological findings to severity of asthma, which included 138 children presenting from cough equivalent asthma to severe asthma. Results indicated that abnormalities occurred in 27 to 36% of patients but with lack of relation to severity of asthma.22

A study has indicated that bronchial hyperresponsiveness to methacholine reflects the severity of asthma. In that study, the hyperresponsiveness was greater in asthmatic patients with infectious sinusitis than in those without sinusitis, but it improved after treatment of sinusitis. The authors also observed that symptoms decreased with appropriate response of sinuses to clinical therapy of sinusitis.23

Our results showed that in atopic patients, mainly asthmatic patients (21%), there is a propensity to severe lesion to paranasal mucosa, but which does not result in more severe asthma or airway obstruction. CT scores of zero were found in only 2 of 33 (6%) asthmatic patients. These findings suggest that involvement of paranasal mucosa is common in asthma patients asymptomatic for infectious sinusitis without resulting in deterioration of asthma. However, our population included only patients with mild and moderate asthma, since they comprise most of the cases being followed-up at outpatient clinics.24 Moreover, we excluded patients who were administered systemic corticoid for treatment of severe asthma.

The disagreement found in the literature regarding the relationship between sinusitis and asthma in children can be attributed to failures in planning, to randomization, and to absence of control groups; moreover, there is a lack of uniform diagnostic criteria and of considering the variability of clinical presentations of asthma in children.25

Newman et al., in a study on adults with chronic sinusitis, found an association between asthma and eosinophilia in cases with severe involvement of the paranasal mucosa indicated by CT scans. Eosinophilia was the determining factor for the severity of mucosal lesion, even in nonatopic patients.16

We observed an association between severity of mucosal lesion indicated by CT scan and peripheral eosinophilia in atopic patients asymptomatic for sinusitis (with asthma and/or allergic rhinitis). This suggests that eosinophils participate in determining severity of lesions, especially in the case of asthmatic patients.

Recently, it is possible to find in the literature the concept of continuity of airways; in this sense, inflammatory processes on the upper airways can both affect the lower airways or be a consequence of lower airway inflammatory processes. This concept is based on histological findings of nasal and endobronchial biopsies of asthmatic patients that indicate extensive infiltration of paranasal tissue with eosinophils much similar to that found in asthma.26,27

The nasal mucosa is a site that is easy to access for the study of inflammatory processes. The fluid from nasal washings can reflect the intensity of the inflammatory process and provide a parallel between symptoms of the upper and lower respiratory tracts.9 The simplified QCNS can be applied to clinical investigations since it quantitates cellularity of nasal mucosa. However, this test still lacks standardization, which makes it more difficult to compare study results.11
We observed that cellularity of nasal fluids could not be used to differentiate patients with higher CT scores. We found percentages of neutrophilia in the nasal fluids of controls, but not absolute neutrophilia. However, these values of neutrophilia were lower than those observed in virus-induced asthma exacerbations. The presence of neutrophils in nasal fluids can be used to diagnose infectious rhinosinusitis, but neutrophilia can also be found in nasal secretion of normal individuals.

Eosinophilia of nasal secretion helped to differentiate asthmatic patients with more severe involvement of the mucosa indicated by CT scan even from the subgroup of patients with less severe lesions.

Noah et al. did not observe a difference in the level of eosinophilia in nasal fluid when comparing allergic, nonasthmatic patients with allergic, asthmatic patients who were all asymptomatic during examination.

Others have observed that inflammatory and structural cells are involved in the production of cytokines and inflammatory mediators in the respiratory mucosa of allergic and asthmatic patients; these cells maintain chronic inflammation and airway hyperresponsiveness.

The concentration of albumin in nasal fluid is one of the parameters for vascular permeability. In our population, the nasal fluid concentrations of albumin were greater in the group of asthma and allergic rhinitis patients (Group I) with severe lesion indicated by CT scan. Only the eosinophils count was proportional to the concentration of albumin, which may reflect a relation between increase in vascular permeability and influx of inflammatory cells, chiefly of eosinophils.

Noah et al. observed higher IL-8 levels in nasal fluid of asthmatic patients in comparison to that of allergic, nonasthmatic and of nonatopic patients (all asymptomatic during examination). Moreover, increased IL-8 levels have been observed in nasal aspirates from children during the virus-induced asthma exacerbations, independently of atopy.

In addition to the differences in methods and population of these studies, our patients were being administered oral theophylline (low dosage) and bronchodilator on demand. The use of oral theophylline may have affected the assessment of mediators in nasal fluids. Naclerio et al. showed that theophylline reduces the release of histamine and other mediators, which can be a result of the effect of xanthines on the activation of mast cells/basophils.

Eosinophilic infiltration of the respiratory mucosa is characteristic of allergic rhinitis and asthma; and so is the release of citotoxic proteins derived from their granules, such as ECP and major basic protein (MBP). Lesions to the respiratory epithelium in asthmatic patients are proportional to eosinophilic infiltration and release of ECP and MBP. Asthmatic patients present significantly higher levels of IL-8 and ECP in nasal washings in comparison to atopic, nonasthmatic patients and to normal individuals.

We observed a positive correlation between CT scores, eosinophils count, and concentration of ECP in nasal fluids. In this sense, more severe involvement of the mucosa indicated by CT scans of facial sinuses was associated with both eosinophilia and activation of eosinophils in nasal fluids; this suggests that lesion to the paranasal mucosa is secondary to eosinophilic inflammation.

Our data indicate that asthmatic patients asymptomatic for sinusitis present more severe involvement of the paranasal mucosa, which may suggest that sinusitis and asthma are related to the same inflammatory process at different levels of the respiratory tract.

Though our patients did not present clinical status of sinusitis, we cannot confirm that they did not have infection of the paranasal sinuses. However, the radiological diagnosis of sinusitis in allergic patients should not be used for indication of prolonged treatment with antibiotics. In this sense, it is important to consider that the involvement of the mucosa of the paranasal sinuses can occur together with allergic, inflammatory response of the respiratory airways instead of with an infectious process. The practical implications of our results involve the need for finding an adequate treatment for respiratory allergies in atopic patients before the investigation through imaging for infectious sinusitis, and before the indication of prolonged use of antibiotics in these patients.

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References