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ORIGINAL ARTICLE

NASAL MUCOSA CELLULAR INFILTRATES IN DIFFERENT PHENOTYPES OF ASTHMATIC CHILDREN: A BRUSH BIOPSY STUDY

By

Malak Shaheen,[1] Manal Salman,[2] Mohamed Elkhouly[3]

[1]Pediatric Department, [2]Pathology Department, Ain Shams Faculty of Medicine, [3]Pediatric Department, Banha Training Hospital, Egypt

Correspondence to: Malak Shaheen, Email: childshaheen@yahoo.com

Background: *Little is known about the cellular infiltrates in nasal mucosa of asthmatic children subtypes.*

Aim of the work: *This study was set up to compare the nasal cellular infiltrates using brush biopsy specimens from asthmatic children of different severities and with or without allergic rhinitis.*

Methods: *Thirty asthmatic children (15 children with allergic rhinitis and 15 children without allergic rhinitis) and 10 age-matched healthy control children were evaluated. All included children were subjected to the following; full medical history, thorough clinical examination, basic spirometric FEV_1 (forced expiratory volume in 1 second), nasal mucosal brush biopsies and assessment of total serum immunoglobulin (Ig) E.*

Results: *A significantly higher numbers of nasal mucosal eosinophils and neutrophils were detected in all asthmatic children compared to controls ($p < 0.01$ and $p < 0.05$ respectively) with best cut off value 7 cells/HPF and 9.5 cells/HPF respectively. Nasal cellular infiltrates discriminate asthma severity and presence of concomitant allergic rhinitis within asthmatic subgroups with cut off values. Eosinophils and neutrophils best cut off values between mild and moderate asthmatics are 17.5 cells/HPF and 9.5 cells/HPF respectively. Eosinophils and neutrophils best cut off values between moderate and severe asthmatics are 50.5 cells/HPF and 52 cells/HPF respectively. Presence of concomitant allergic rhinitis was defined with eosinophils and neutrophils nasal mucosal infiltrates more than 47.5 cells/HPF and 45 cells/HPF respectively.*

In conclusion: *These results show that the nasal cellular infiltrates of asthmatic children differ from healthy controls and within asthmatics subtypes. Cut off values of nasal cellular infiltrates may aid to predict asthma severity and/or presence of concomitant allergic rhinitis.*

Keywords: *Asthma, children, phenotypes, nasal mucosa, cellular infiltrates.*

INTRODUCTION

Both nasal mucosa and bronchial mucosa are elements of a "united airway".(1) There is no doubt that asthma is one of the most important chest diseases in pediatrics which is defined as chronic, inflammatory condition of the airways.(2)

The disturbances of upper and lower airways frequently coexist.(1) As it represents a single airway, a reciprocal diagnosis, follow up and treatment of both upper and lower respiratory disease is essential.(3)

The present study reports on the hypothesis that cellular infiltrates in the nasal mucosa of asthmatic children could discriminate different asthma severities and phenotypes.

To understand the dynamics of the development of childhood asthma disease it is of paramount importance to study all cell types in the nasal mucosa of asthmatic children with different presentations. The present study was performed to obtain greater insight into measurable cellular changes within different asthmatic subtypes.

PATIENTS AND METHOD

Subjects:

This study was performed with the approval of the Medical Ethics Committee of Ain Shams University – Cairo, Egypt.

In the period from May to December 2008, asthmatic children who attended - Ain Shams Pediatric Hospital - Specialized Pediatric Chest Clinic - were asked to participate in the study. Healthy children were recruited from families of admitted children to the hospital. The parents or guardians of all the included children gave their written informed consent to participate in the study.

The total group consisted of 40 children; 30 asthmatic children (19 males and 11 females) and 10 healthy controls (6males and 4 females) with age range 5-12 years (Mean ±SD = 6.92 ± 2.013) for asthmatics and 5-12 years (Mean ±SD = 7.32 ± 1.735) for healthy controls.

Study design:

All the participating children were subjected to:

1- Medical history taking: The medical history of all children was taken by a physician on the basis of an extensive questionnaire. Data were collected on symptoms and signs of the children's disease(s), medication, rhinitis, asthma, eczema and family history with respect to allergy.

2- Careful general and local chest examination.

3- Investigations:

- Pulmonary function tests were measured using Spirobank, MIR Italy pocket spirometer to detect; forced expiratory volume in 1st second (FEV_1) of the children. It was expressed as a percentage of predicted for age and sex.
- A blood sample was taken to measure total serum IgE. This was carried out using ELISA technique, using the kit provided by BioCheck, Inc (Foster City, CA 94404 U.S.A).

Children were included in the asthmatic group (Table 1) if they fulfilled the set of inclusion criteria of GINA guidelines 2008 signs and symptoms of childhood asthma.[4] Allergic rhinitis of asthmatic children was diagnosed according to the criteria of ARIA study 2008 update.[5]

Children were included in the control group if they had no symptoms of allergic disease together with a low total IgE for their age (<10 IU.yr of age-1) and a negative family history for atopic disease.

Table 1. Classification of the included asthmatic children.

Asthmatic Children	With allergic rhinitis		Without allergic rhinitis		Total
Mild Persistent	4	26.7%	4	26.7%	8
Moderate	9	60%	8	53.3%	17
Severe	2	13.3%	3	20%	5
Total	15	100%	15	100%	30

Children were excluded from both groups; when their age was less than 5 years (to be able to perform spirometry), parents did not give an informed consent, when the child had purulent rhinitis at the time of biopsy and when systemic or topical corticosteroids had been used in the 2 months prior to biopsy.

Specimen Collection:

Nasal mucosal specimens were taken via disposable nasal brushes by rubbing slowly and carefully in a circular manner against the middle one third of the inferior turbinate;[6] they were applied quickly to slides to be fixed in absolute alcohol (100% concentration).

Fig 1. Nasal brush.

Staining Procedure and Light Microscopic Evaluation:

They were stained by Eosin and Hematoxilin stains to be examined by high power field microscope at 400 magnitudes for detection of inflammatory cellular infiltrates (eosinophils, neutrophils) and denuded epithelial cells. The total number of isolated cells was determined separately by two independent researchers.

Statistical analysis:

As slides were counted blindly by two independent researchers, inter observer reliability was determined. This was carried out by calculating the Spearman rank correlation. For each cell type a strong correlation was found ($rs > 0.85$, $p < 0.05$). The statistical analysis of cell numbers was performed using SPSS (version 11 for Windows; SPSS, Chicago, IL, USA). The results were collected and tabled to undergo statistical analysis including:

1- Descriptive statistics:

- Quantitative data: in the form of mean and standard deviation.
- Qualitative data: in form of number and percentage.

2-Analytical statistics:

Comparison of different variables in various groups was done using Mann Whitney test for nonparametric variables. Comparisons of multiple subgroups were done using Kruskall Wallis test for nonparametric variables. Chi-square (χ^2) test was used to compare frequency of qualitative variables among the different groups. Spearman's correlation test was used for correlating non-parametric variables. Receiver Operator Curve (ROC) was drawn to calculate best cut off values discriminating different phenotypes of asthmatic children. For all tests p value <0.05 were considered significant, p value <0.01 were considered highly significant and p value >0.05 were considered non significant.

RESULTS

Biopsy procedure:

All the participating children in this study had no adverse effects after the brush biopsy. The procedure was easy, rapid and painless. Neither proceeding events nor post-biopsy follow up was needed. The yield of the procedure was 100% successful with no exclusion or repetition of biopsies.

General description:

All biopsy specimens showed evidence of the characteristic airways columnar epithelium containing goblet cells. However; this denuded epithelium was more evident in asthmatics compared to healthy controls. Scattered inflammatory cells infiltrate (mainly eosinophils and neutrophils) was reported much more in asthmatic children compared to controls (Fig 2).

Fig 2. Light microscopic pictures of asthmatics' brush specimen smears.

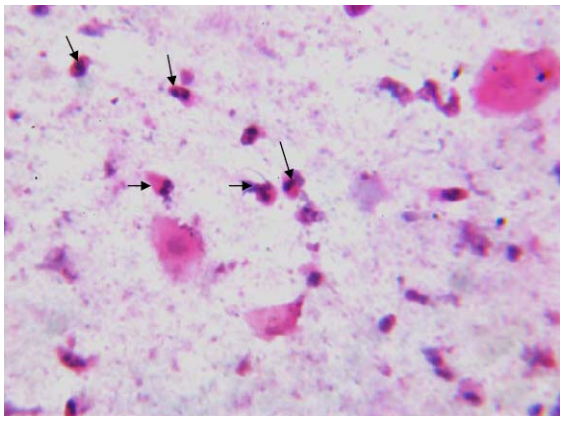


Fig 2a. This a nasal smear from an asthmatic child showing many eosinophils (Arrows). (H&E X400).

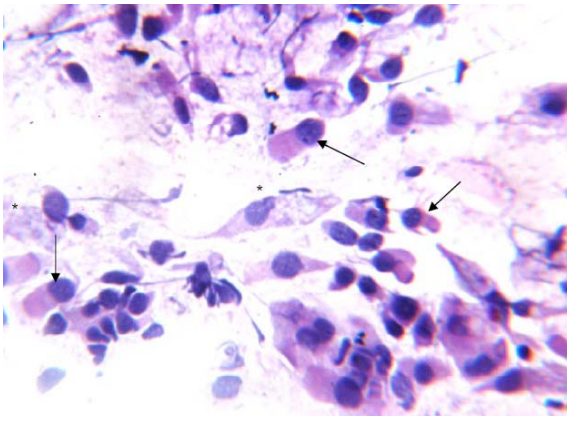


Fig 2b. This a nasal smear from an asthmatic child showing increased denuded respiratory columnar epithelial cells (Arrows) and goblet cells (*). (H&E X450)

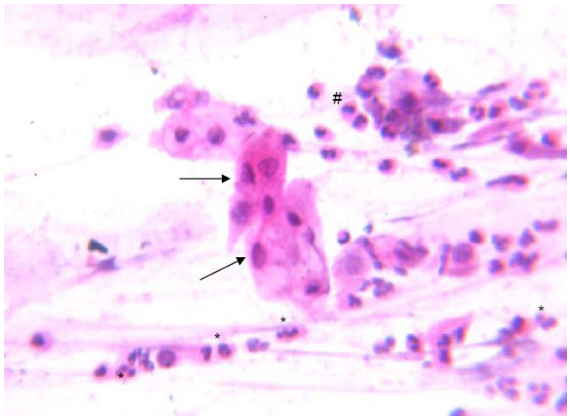


Fig 2c. This a nasal smear from an asthmatic patient showing denuded squamous epithelial cells (arrows) surrounded by neutrophils(*) and eosinophils (#) (H&E X450)

Inflammatory cellular infiltrate in the nasal mucosa biopsies:

Eosinophils and neutrophils were the predominant isolated inflammatory cellular infiltrate in all the specimens with higher concentrations in asthmatic children compared to controls (Fig. 3). However; the discriminating hall mark cellular infiltrates in asthmatics were eosinophils (almost absent in controls and predominant cellular infiltrate in asthmatic children).

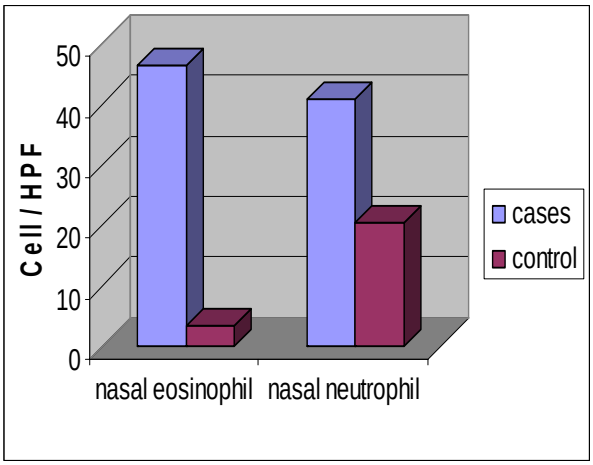


Fig 3. Bart chart of nasal eosinophils and neutrophils (mean values) results.

Eosinophilic counts in nasal brush biopsy of asthmatic children were the robust evidence of the airway inflammation severity and as a tool to discriminate different phenotypes of asthma severity (mild, moderate and severe) with ($p<0.0001$) Table 2.

Also, eosinophilic counts in nasal brush biopsy of asthmatic children showed a significant negative correlation with the degree airways obstruction as evident by (FEV_1) (Fig. 4a) and the degree of atopy as evident by a significant positive correlation with total serum IgE levels (Fig. 4b).

On the other hand; neutrophilic counts in nasal brush biopsy of asthmatic children were only evident with moderate and severe asthmatic children (mild asthmatics were indifferent from controls) Table 3. This makes neutrophilic count a less sensitive marker for asthmatic children.

Fig 4. ***Correlations of nasal eosinophilic counts in asthmatic children.***

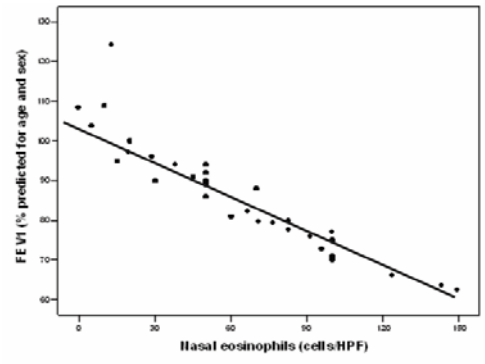


Fig 4a. Correlation between FEV1 and nasal eosinophilic counts of asthmatic children.

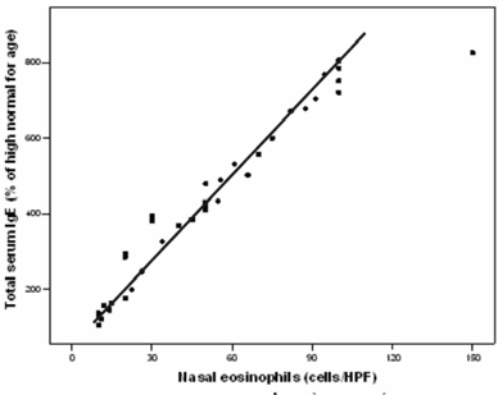


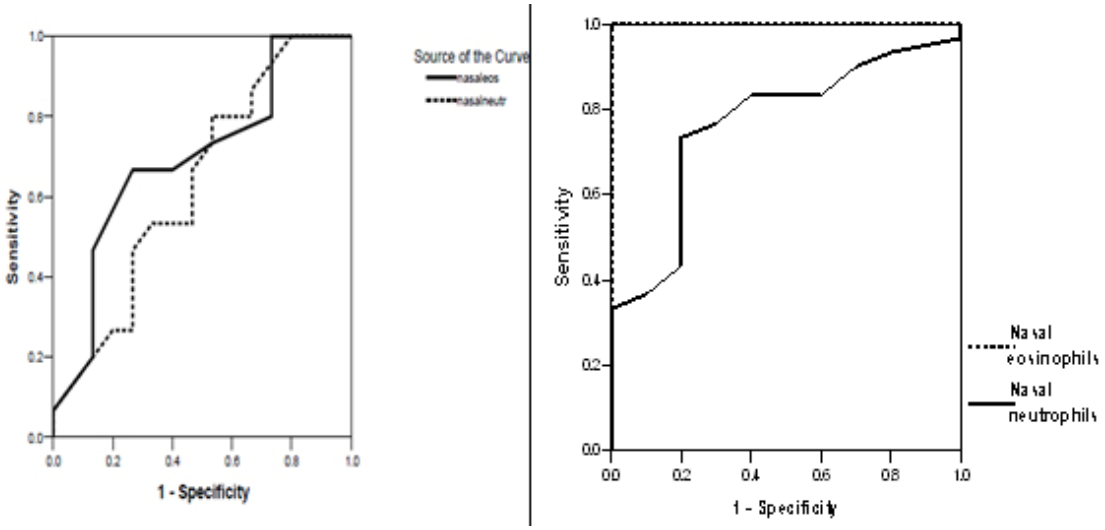
Fig 4b. Correlation between total IgE (% of high normal) and nasal eosinophilic counts of asthmatic children.

Asthmatics with nasal allergy:

Presence of concomitant allergic rhinitis manifestation in asthmatic children was discriminated in the study by nasal specimens with significantly higher eosinophilic and neutrophilic cell counts. Also total serum IgE levels were significantly higher if allergic rhinitis was manifested (two atopic diseases versus one).

Spirometric lung functions showed no significant difference regarding FEV_1 if concomitant allergic rhinitis is evident Table 4.

Receiver operating curves (ROC) were drawn to plot best cellular infiltrate cut off values to diagnose pediatric asthma and to differentiate pediatric asthma phenotypes (Fig. 5).



Asthmatics with versus without allergic rhinitis. ***Asthmatics versus Controls.***

Fig 5. ROC curves.

Eosinophilic counts is evident to be the most sensitive and specific marker to discriminate asthmatic children from healthy controls with best cut off value = 7 Cells/HPF (Specificity 100%, Sensitivity 100% and AUC 1). Similarly; eosinophilic count was the most reliable marker to discriminate mild from moderate from severe pediatric asthma with highest specificity, sensitivity and AUC Table 5.

However; this discrimination is less effective to discriminate pediatric asthma with and without allergic rhinitis (specificity 84%, sensitivity 79% and AUC 0.69)

Neutrophilic counts showed less specific and sensitive cut off values to discriminate pediatric asthma phenotypes Table 5.

Data of total serum IgE in the study groups:

Levels of total serum IgE were expressed as a percentage (%) of the maximum limit of the normal range for age (to standardize values according to age groups).[7] The mean and standard deviation of these levels were used statistically. Levels of total serum IgE in asthmatic children (412+120) were significantly higher compared to the healthy control children (66+17) with ($p<0.0001$).

The highest levels were recorded in children with the more severe disease (141+23 for mild asthmatics, 432+94 for moderate asthmatics and 776+42 for the severe asthmatics). Also asthmatic children with allergic rhinitis showed statistically significant higher levels compared to those without allergic rhinitis Table 5.

These high levels showed persistently a significant positive correlation with the nasal mucosa eosinophilic cellular infiltrate load (Fig. 4b).

Table 2. Mean values of nasal eosinophils (Cell/HPF).

		Mild No.=8	Moderate No.=17	Severe No.=5	Control No.=10
Mean ± SD		12.75 ± 3.495	43.82± 16.82	110 ± 22.36	3.40 ± 1.430
Mild	z		-3.903	-3.586	-3.588
	p		.000 (high sig.*)	.000(high sig.)	.000(high sig.)
Moderate	z			-3.366	-4.137
	p			.000(high sig.)	.000(high sig)
Severe	z				-3.599
	p				.000(high sig)

*High sig. = highly significant.

Table 3. Mean values of nasal neutrophils (Cell/HPF).

		Mild No.=8	Moderate No.=17	Severe No.=5	Control No.=10
Mean ± SD		14.25 ± 8.303	51.12± 23.71	90.15 ± 12.24	20.60± 11.998
Mild	z		-2.801	-2.94	-2.71
	p		.004 (sig.*)	.002 (sig.)	.06 (Non sig.#)
Moderate	z			-2.204	-2.988
	p			.004(sig.)	.044(sig.)
Severe	z				-.873
	p				.002 (sig.)

* Sig. = significant, # Non sig. = non significant.

Table 4. Comparison between asthmatic children with and without allergic rhinitis.

	Asthmatic without allergic rhinitis No.=15	Asthmatic with allergic rhinitis No.=15	z	P	Sig.
	Mean ± SD				
Age	6.60 ±1.88	7.23 ± 1.88	-1.25	.217	Non sig.#
Nasal eosinophil cell/HPF.	46.48± 12.40	56.73 ± 18.45	-2.03	.047	Sig.*
Nasal neutrophil cell/HPF.	47.47±17.66	56.47 ± 19.90	-1.24	.051	Sig.
Total IgE %of high normal for age	351.00±88.25	473.73 ± 128.04	-2.09	.037	Sig.
FEV1% Percentage of predicted for age and sex	94.20 ± 18.01	84.47 ± 9.24	-1.22	.233	Non sig.

* Sig. = significant, # Non sig. = non significant.

Table 5. ROC curve results of cellular infiltrate best cut off values.

Groups	Variable	Best cut off (Cells/HPF)	Specificity %	Sensitivity %	Area Under the Curve (AUC)
Asthmatics vs* Controls	E**	7	100	100	1
	N#	9.5	80	73	0.76
Mild vs* Moderate Asthmatics	E	17.5	98	100	0.98
	N	9.5	85	85	0.84
Moderate vs* Severe asthmatics	E	50.5	100	100	1
	N	52	82	75	0.75
With vs* without allergic rhinitis	E	47.5	84	79	0.69
	N	45	70	72	0.63

Vs*= versus, E**= Eosinophils, N#=Neutrophils.

DISCUSSION

The initial hypothesis in the present study was that cellular infiltrates in the nasal mucosa of asthmatic children could discriminate different asthma severities and phenotypes. The results show that eosinophilic counts are the most sensitive and specific marker to diagnose and differentiate pediatric asthma phenotypes with measurable cut off values Table 5.

To the authors' knowledge no data has yet been produced on nasal mucosa cellular infiltrates cut off values to diagnose and discriminate pediatric asthma different phenotypes using the simple brush biopsy technique. Current knowledge on this subject is based on data collected primarily from adults and to less extent obtained from children describes only qualitatively the pathological changes of nasal mucosa accompanying asthma without any quantitative measurable solid points for assessment.[5-8]

In early childhood it is not always easy to prove that a child is asthmatic, because of the absence of a clear-cut medical history and the lack of fully reliable laboratory tests to discriminate different childhood asthma phenotypes.

The nasal cavity, paranasal sinuses and lungs are considered separate organs of the respiratory tract. However, a growing body of scientific evidence and clinical observations now link the upper and lower airways as the "united airway".[1-3] Evidence includes: similar histology and physiology; near identical immune and clinical responses to allergens and the coexistence of allergic rhinitis or rhinosinusitis and asthma.[7]

Also studies, which link the upper and lower airway responses to allergens, support the concept that an allergic stimulus in one organ system, either the nose or lungs, results in a systemic allergic inflammatory response which affects the other.[8]

For example, allergen challenge of the nose increases the allergic eosinophilic inflammation of the bronchi,[9] and bronchial allergen challenge enhances the allergic inflammation in the nose.[10]

Similarly; the rhinitis (rhinosinusitis) and asthma association is reinforced by the observations that the treatment of one of these respiratory organs benefits the other. Not only does intranasal beclomethasone reduce the symptoms of allergic rhinitis, it improves asthma symptoms and BHR and lowers asthma medication requirements and ER visits.[11]

Brushing technique of nasal mucosa of asthmatic children was done to obtain a 'Cytology brush biopsy'. With this, a cytology brush is rubbed against the airway surface in the nose in a fashion similar to the one used in the diagnosis of primary ciliary dyskinesia (PCD). Cell yield is usually respiratory epithelium and will vary greatly depending, in part, on the size of brush used and partly on the underlying pathology.[12-14] So, for quantitative assessment of such biopsies, control subjects must be included and/or a standardized fixed technique must be implicated.

Several groups have reported safety, success and easy implication of such nasal non bronchoscopic biopsy alternative to sample airway mucosal cells, which may have important implications in terms of time and resources.[15-18]

With basic stains of nasal mucosa brush biopsy, such as haematoxylin and eosin (H&E), many histological structures can be visualized.[6] If the biopsy is of sufficient quality, the ciliated respiratory epithelium, together with mucus-containing cells such as goblet cells and inflammatory cellular infiltrate will be easily recognizable.[19]

Many literature resources emphasize the importance of airways oesinophilic infiltrates as a hall mark and evidence of asthma pathogenesis.[4,5,20-23] Recently; neutrophilic cellular infiltrates started to be incriminated as an important trigger for chronic airways inflammation of asthma.[24-25]

In this study, eosinophilic counts in nasal mucosa brush biopsy were a sharp marker to differentiate all childhood asthma subtypes according to severity with clear cut off values. This sharp discrimination was less clear using neutrophilic counts, however the evidence of higher neutrophilic cellular infiltrates pointed to more severe and less controlled asthmatic child.

The association between airway neutrophilic cellular infiltrates and severe asthmatics was addressed also by many studies [24,26] and sometimes entitled separately as "Neutrophilic asthma" phenotype which may not be assigned to all asthmatic subjects.[27]

The association between allergic rhinitis and asthma is recognized as a manifestation of atopy.[28,29] The World Health Organization sponsored the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines in 2008 as an evidence-based document linking both allergic rhinitis to asthma.[5] Many children with allergic rhinitis have concomitant asthma and most asthmatics have coexistent allergic rhinitis.[4]

However, the hypothesis to discriminate pediatric asthma

phenotypes using nasal mucosa brush biopsies does not appear to be valid in the present study for asthmatic children with and without allergic rhinitis. The explanation of such phenomenon may be existence of other cofactors which may affect results; like adjustment of asthma severity during comparison. Nevertheless, it was quite difficult to perform severity adjustment in this study because of the small number of the studied children.

Also in this small case control study, it was a bit difficult to assess the inflammatory cellular infiltrate dynamics in relation to different asthma therapeutics and/or acute exacerbations. These important cofactors must be studied with further longitudinal follow up studies on larger number of children.

In conclusion, the nasal mucosa of asthmatic children differs from normal healthy children and differs according to asthma severity. Inflammatory cellular infiltrates especially eosinophilic counts can quantitatively effectively discriminate pediatric asthma phenotypes with high sensitivity and specificity. Furthermore, the nasal brush biopsy technique is a safe, easy, non invasive and cheap method for future studies in young children with asthma. However; more studies are needed to standardize this technique on larger number of children of different phenotypes with adjustments of cofactors.

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REFERENCES

1. Bousqet J, Jacquot W, Vingola AM. Allergic rhinitis a disease remodeling the upper airway. J Allergy Clin Immunol. 2004;113:43-9.

2. Barbato A, Turato G, Baraldo S. Airway inflammation in childhood asthma. Am. J. Respir. Crit. Care Med. 2003;168:798–803.

3. Bacharier LB, Boner A, Carlsen KH. The European Pediatric Asthma Group. Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. Allergy. 2008:63:5–34.

4. Global Strategy for Asthma Management and Prevention. The Global Initiative for Asthma (GINA). 2008. http://www.ginasthma.com/GuidelinesResources. Accessed. 2008.

5. Bousquet J, Khaltaev N, Cruz AA. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update (in collaboration with the World Health Organization, GA2LEN and AllerGen). Allergy. 2008:63:8–160.

6. Lane C, Burgess S, Kicic A. The use of non-bronchoscopic brushings to study the pediatric airway. Respir Res. 2005;6:53.

7. Pohunek P, Warner JO, Turzíková J. Markers of eosinophilic inflammation and tissue re-modeling in children before clinically diagnosed bronchial asthma. Pediatr Allergy Immunol. 2005;16:43-51.

8. Brand PLP, Baraldi E, Bisgaard H. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach (Task Force). Eur Respir J. 2008;32:1096–110.

9. Merigo F, Benati D, Piacentini G. The ultra structure of nasal mucosa in children with asthma. Ultra structural pathology. 2007;26:293-8.

10. Shaheen MA, Fawaz SA, Salman MI. The ultrasturcture of nasal mucosa in asthmatic children with and without allergic rhinitis. Egy J Ped. 2005;22:115-31.

11. Fuhlbrigge AL, Adams RJ. The effect of treatment of allergic rhinitis on asthma morbidity, including emergency departments visits. Curr Opin Allergy Clin Immunol. 2003;3:29-32.

12. Jeffery PK, Davies JC. Increased Airway Smooth Muscle Mass in Children with Asthma, Cystic Fibrosis and Bronchiectasis. Am J Respir Crit Care Med. 2008;177:837-43.

13. Saglani S, Payne DN, Zhu J. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med. 2007;1:176:858-64.

14. Hilliard TN, Regamey N, Shute JK. Airway remodelling in children with cystic fibrosis. Thorax. 2007;62:1074-80.

15. Regamey N, Hilliard TN, Saglani S. Quality, size, and composition of pediatric endobronchial biopsies in cystic fibrosis. Chest. 2007;131:1710-7.

16. Molina-Teran A, Hilliard TN, Saglani S. Safety of endobronchial biopsy in children with cystic fibrosis. Pediatr Pulmonol. 2006;41:1021-4.

17. Saglani S, Malmström K, Pelkonen AS. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med. 2005;171:722-7.

18. Payne DN, Qiu Y, Zhu J, et al. Airway inflammation in children with difficult asthma: relationships with airflow limitation and persistent symptoms. Thorax. 2004;59:862-9.

19. Saglani S, Payne DN, Nicholson AG. The safety and quality of endobronchial biopsy in children under five years old. Thorax. 2003;58:1053-7.

20. Payne DN, Rogers AV, Adelroth E. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med. 2003;167:78-82.

21. Payne D, McKenzie SA, Stacey S. Safety and ethics of bronchoscopy and endobronchial biopsy in difficult asthma. Arch Dis Child. 2001;84:423-6.

22. Bush A, Pohunek P. Brush biopsy and mucosal biopsy. Am J Respir Crit Care Med. 2000;162:S18-22.

23. Kikuchi S, Nagata M, Kikuchi I. Association between neutrophilic and eosinophilic inflammation in patients with severe persistent asthma. Allergy Immunol. 2005;137:7-11.

24. Lane C, Burgess S, Kicic A. The use of non-bronchoscopic brushings to study the pediatric airway. Respir Res. 2005;8:6:53.

25. Togias A. Rhinitis and asthma: evidence for respiratory system integration. J Allergy Clin Immunol. 2003;111:171-83.

26. Yoshihara S, Yamada Y, Abe T. Association of epithelial damage and signs of neutrophil mobilization in the airways during acute exacerbations of pediatric asthma. Clin Exp Immunol. 2006;144:212–16.

27. Simpson JL, Scott R, Boyle MJ. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology. 2006;11:54-61.

28. Katelaris CH. Allergic rhinitis and asthma: epidemiologic evidence for the link. Clin Exp All Rev. 2003;3:5-8.

29. Bousqet J, Vignola AM, Demoly P. Links between rhinitis and asthma. Allergy. 2003;58:691-706.