

Short communication

Sputum neurokinin A in Egyptian asthmatic children and adolescents: relation to exacerbation severity

Background: Neurogenic inflammation may participate in the development and progression of bronchial asthma. The molecular mechanisms underlying neurogenic inflammation are orchestrated by a large number of neuropeptides including tachykinins such as neurokinin A (NKA) and substance P. Tachykinins are secreted from sensory airway nerves and inflammatory cells after allergens exposure. In clinical practice, assessment of airway inflammation is difficult. Therefore, detection of biological markers of airway inflammation in sputum might offer help for proper monitoring of asthma severity.

Aim of the study: We aimed to measure sputum NKA in relation to acute asthma exacerbations of varying severity.

Methods: Sputum NKA was measured by enzyme-linked immunosorbent assay in 24 children and adolescents during and after acute asthma exacerbation and 24 healthy matched controls.

Results: Sputum NKA was significantly higher in asthmatic patients during acute exacerbation than controls [217.5 (284) vs 10 (7) ng/ml, $P < 0.001$]. When patients with acute asthma exacerbation were followed-up till remission, sputum NKA levels decreased significantly, but they remained significantly higher than controls. Sputum NKA levels were significantly higher in severe than moderate and in moderate than mild exacerbations, and was negatively correlated to peak expiratory flow rate ($r = -0.9$, $P < 0.001$). Sputum NKA had significant positive correlations to eosinophil counts in blood and sputum ($r = 0.6$, $P < 0.001$ and $r = 0.7$, $P < 0.001$ respectively).

Conclusions: Sputum NKA is up-regulated during acute asthma exacerbation and it positively correlates to its severity. Thus, NKA may aid in objective classification of the exacerbation severity. In addition, NKA may be a target for new asthma therapy.

**G. A. Mostafa¹, S. M. Reda¹,
M. M. Abd El-Aziz², S. A. Ahmed¹**

Departments of ¹Pediatrics and ²Clinical Pathology,
Faculty of Medicine, Ain Shams University, Cairo,
Egypt

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Gehan Mostafa
9 Ahmed El-Samman Street off Makram Ebad
Nasr City
Cairo
Egypt

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Neurogenic inflammation may participate in the development of chronic inflammatory airway diseases such as bronchial asthma. The molecular mechanisms underlying neurogenic inflammation are orchestrated by a large number of neuropeptides mainly including tachykinins, such as neurokinin A (NKA) and substance P (SP) and calcitonin gene-related peptide (1). Tachykinins belong to a family of neuropeptides, which are released from nonadrenergic, noncholinergic excitatory nerves of airways (2) after exposure to allergens. They are also produced by inflammatory cells such as eosinophils (3, 4).

Neurokinin A and SP have potent effects on broncho-motor tone, airway secretions and bronchial circulation. In view of their potent effects on the airways, tachykinins have been put forward as possible mediators of asthma, and tachykinin receptor antagonists are a potential new class of antiasthmatic medications (3, 5–7).

Assessing biological markers of airway inflammation for proper diagnosis, monitoring and treatment of bronchial asthma is essential (8). Sputum induction is a noninvasive test that monitors airway inflammation. Sputum markers tend to be more sensitive than blood tests when assessing airway inflammation (9).

With this background, we were stimulated to investigate sputum NKA levels in children and adolescents with acute asthma exacerbations of varying severity.

Methods

Study population

This case-control, follow-up study was conducted on 24 asthmatic children and adolescents recruited from the Pediatric Allergy and Immunology Unit, Children's

Hospital, Ain Shams University, over a period of 9 months from the beginning of October 2006 to the end of June 2007. They were 16 males and eight females, their ages ranged between 6 and 16 years with a mean age of 10.1 ± 3.7 years. Patients were subdivided according to the severity of acute asthma exacerbation into three equal subgroups: mild, moderate and severe.

The diagnosis of asthma and the severity of acute exacerbations were based on clinical and functional assessment using peak expiratory flow rate (PEFR; 10). Patients with acute exacerbations were followed up for 2–3 weeks until remission, then follow-up blood and sputum samples were collected.

Twenty-four healthy-matched children without allergic manifestations were studied as controls. They were 16 males and eight females, their ages ranged between 6 and 16 years with a mean age of 10.3 ± 3.9 years.

The local Ethical Committee of the Faculty of Medicine, Ain Shams University approved this study. In addition, an informed written consent of participation in the study was signed by the parents of the studied subjects.

Study measurements

Measurement of PEFR. Mini-Wright Peak Flow Meter (Clement Clarke International Ltd, Harlow, England) was used.

Sputum induction. Sputum was induced only when it could not be produced spontaneously. Adequate sputum samples could be obtained spontaneously in 14 asthmatic and 14 control subjects. The remaining subjects (10 asthmatic and 10 control subjects) were premedicated with two puffs inhaled salbutamol (200 µg) 30 min before sputum induction. To minimize contamination with saliva and postnasal drip, subjects were asked to rinse their mouths and blow their noses before induction. Subjects inhaled 3% hypertonic saline solution aerosols generated by Farmasol compressor nebulizer. Sputum induction was stopped if the PEFR% predicted fell by $\geq 15\%$ (11).

Measurement of sputum eosinophils. They are expressed as percentage of leucocytes in sputum. Patients were considered to have elevated sputum eosinophils% if they were above 2%.

Assessment of sputum NKA. This was performed by a person blind to the clinical conditions of the patients using enzyme-linked immunosorbent assay (ELISA) technique which is highly sensitivity to NKA. NKA like immunoreactivity was measured using an antibody that has originally been isolated from porcine spinal cord. It shows 100% cross reactivity to NKA with little reactivity to other tachykinins (Peninsula laboratories, 611 Talor wat, Belmont, CA, USA).

Assessment of blood eosinophil count. It was assayed by Coulter counter (Beckman coulter 3microDiff 18, Fullerton, CA, USA).

Serum total IgE assay. It was assayed by ELISA (Eurogenetics; IgE Quantitative, Tessen Derlo, Belgium).

Statistical analysis

The results were analysed by software package (STATVIEW; Abacus Concepts, Inc, Berkley, CA, USA). Sputum data (nonparametric) were presented as median and inter-quartile range. Screening of nonparametric data for differences among three groups was performed using Kruskal–Wallis test. When this was significant, each pairing was examined using Mann–Whitney *U*-test. Spearman's correlation coefficient '*r*' was used to determine the relationship between quantitative variables. For all tests, $P < 0.05$ was considered significant.

The patients were considered to have elevated sputum NKA levels if they were above the highest cut-off value (170 ng/ml) which was the 95th percentile of the non-parametric control values.

Results

The characteristics of the study subjects are shown in Table 1. Patients with acute asthma exacerbation had significantly higher levels of sputum NKA than healthy controls. When patients with acute asthma exacerbation were followed-up till remission, sputum NKA levels decreased significantly, but they remained significantly higher than controls (Fig. 1).

Sputum NKA levels went hand in hand with exacerbation severity. These levels were significantly higher in severe [420 (182) ng/ml] than moderate [217 (75) ng/ml] and in moderate than mild [40 (13.3) ng/ml] exacerbations (Fig. 2). Furthermore, sputum NKA had significant negative correlation to PEFR ($r = -0.9$, $P < 0.001$).

There was nonsignificant difference between sputum NKA levels in induced ($n = 10$) and noninduced ($n = 14$) sputum of both asthmatic children ($P > 0.05$).

Table 1. Basic clinical and laboratory data of asthmatic patients and control group

	Asthmatic patients ($n = 24$)	Control group ($n = 24$)
Age (years)	10.1 ± 3.7 (6–16)	10.3 ± 3.9 (6–16)
Sex (M/F)	16/8	16/8
Duration of illness (years)	7.6 ± 3.6 (1–13)	
Sputum NKA during exacerbation (ng/ml)	217.5 (284)	10 (7)
Sputum NKA during remission (ng/ml)	46 (141)	
Sputum eosinophils %	3 (5.8)	1 (1)
Blood eosinophil count (cell/mm ³)	417.5 (665)	95 (97)
Serum IgE (IU/l) %	197.5 (357.5)	

NKA, neurokinin A.

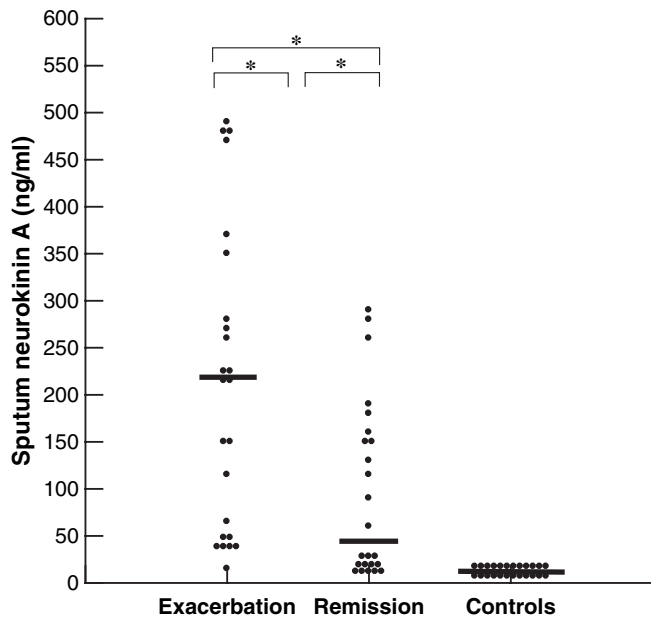


Figure 1. Comparison of sputum levels of NKA between asthmatic patients during exacerbation and remission and controls. Median values are represented as horizontal barr. * $P < 0.01$.

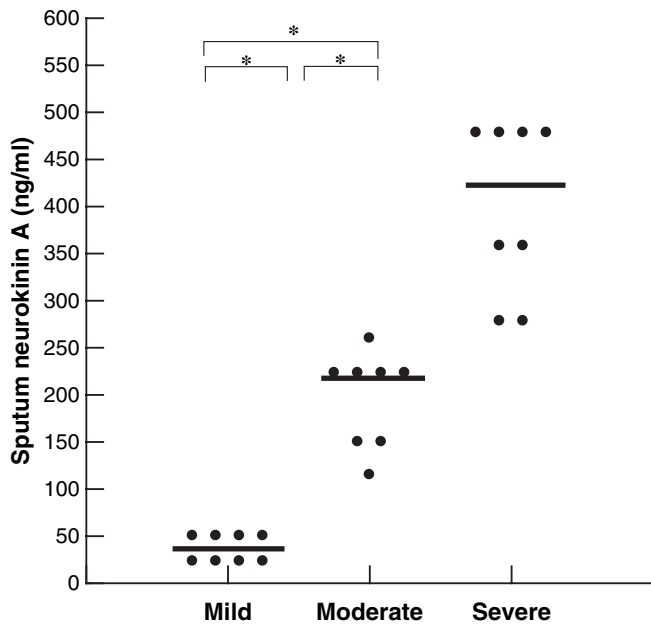


Figure 2. Sputum NKA levels in relation to the severity of acute asthma exacerbation. Mean values are represented as horizontal barr. * $P < 0.01$.

and control subjects ($P > 0.05$). Sputum NKA levels were elevated above the calculated highest cut off value in 95.8% of asthmatic patients during exacerbation (Table 2).

Sputum NKA had significant positive correlation to blood eosinophil counts ($r = 0.6$, $P < 0.001$) and sputum

Table 2. Percentage of patients with elevated levels of neurokinin A and other studied markers for acute asthma exacerbation

Asthma inflammatory marker	All asthmatic patients (n = 24)	Mild exacerbation (n = 8)	Moderate exacerbation (n = 8)	Severe exacerbation (n = 8)
Sputum neurokinin A, %	95.8	87.5	100	100
Sputum eosinophils, %	66.7	50	75	75
Blood eosinophil counts, %	54.2	37.5	62.5	62.5

eosinophils % ($r = 0.7$, $P < 0.001$). In contrast, sputum NKA did not correlate significantly to serum IgE, age of patients and duration of illness ($P > 0.05$).

Discussion

In our series, sputum NKA was significantly elevated in asthmatic patients during exacerbation as compared to controls. These results conform with a recent study which demonstrated increased tachykinins (NKA and SP) levels in induced sputum of asthmatic patients and cough patients with coexistent acid reflux (12). A previous study (13) reported a high concentration of NKA in bronchoalveolar lavage (BAL) in asthmatic subjects and it was the predominant tachykinin in BAL of these subjects. NKA has been shown to induce rapid bronchoconstriction in both asthmatic and nonasthmatic subjects closely resembling the response to muscarinic-3 agonist methacholine (14).

In the present study, sputum NKA correlated positively to the severity of asthma exacerbation as evidenced by the graded increase in its levels that went hand in hand with the degree of exacerbation severity. In addition, it correlates negatively to PEFR ($r = -0.9$, $P < 0.001$).

In 2003, Shang and associates (15) reported elevated plasma levels of NKA in asthmatic children with a significant positive correlation to asthma severity. Therefore, the relationship between NKA and bronchial asthma could be a causal one in which this neuropeptide might not only play a role in the evolution of acute asthma exacerbation, but its levels could also determine its clinical severity. Broad-scale studies are warranted to set cut-off values of NKA for each grade of asthma severity. Indeed, this will allow for a better management of asthma exacerbations.

Inflammatory cell (e.g. eosinophils) derived tachykinins are a major second source of these proinflammatory mediators in bronchial asthma (4). In our series, sputum NKA had significant positive correlations to eosinophil counts in both blood and sputum of asthmatic children during exacerbation. Tachykinins may induce the so-called neurogenic inflammation by recruitment and activation of inflammatory cells like eosinophils (4). Thus, NKA-induced bronchospasm may result from eosinophil chemoattraction.

Although sputum NKA levels sputum NKA of asthmatic patients were significantly decreased during asthma quiescence, yet these levels were still significantly higher than controls. These findings may denote that inspite of apparent clinical quiescence of bronchial asthma, there is underlying inflammation with release of neuropeptides such as NKA from inflammatory cells, which result in airway inflammation. This underscores the importance of clinical examination alone as a guidance of subsidence of asthma exacerbation and highlights the importance of searching for inflammatory markers of bronchial asthma

(e.g. NKA) for objective measurement of asthma quiescence and guidance of proper response to asthma therapy.

In conclusion, sputum NKA is up-regulated during acute asthma exacerbation and it positively correlates to exacerbation severity. Thus, it may play a role in the evolution of acute asthma exacerbation and it may aid in the objective classification of its severity with better management of bronchial asthma. In addition, NKA may be a target for new asthma therapy.

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