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ORIGINAL ARTICLE

Nasal lavage fluid nuclear factor kappa B and cytology in asthmatic children and their correlation with severity and control

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KEYWORDS

Nasal lavage;
NFκB;
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Airway inflammation

Abstract *Background:* Asthma is the most common chronic inflammatory disease in childhood. The relevance of NFκB which is known to be an inflammatory marker in upper airway epithelium and its relation to lower airway inflammation has not been fully studied in childhood asthma.

Aim of study: The study aimed at evaluating the diagnostic value of nasal lavage nuclear factor kappa B and cells as a less-invasive bench-side maneuver and inflammatory biomarkers in asthmatic children and correlating with asthma severity.

Methods: This case-control study recruited 60 asthmatic children from Pediatric Chest Clinic, Children's Hospital; Ain Shams University. Thirty healthy non-asthmatic children-age and sex-matched were included as a control group. Nasal lavage cytology, nasal lavage NFκB and forced expiratory volume in 1 s (FEV1) % of predicted for age and sex were estimated.

Results: Nasal lavage NFκB levels were significantly higher in asthmatics than in controls with a mean of 0.129 ± 0.113 μg/μg nuclear proteins and 0.0176 ± 0.013 μg/μg nuclear proteins, respectively. Nasal lavage NFκB and eosinophil levels were significantly higher with increasing asthma severity and with worsening levels of asthma control. Nasal lavage NFκB showed a sensitivity of 87% and a specificity of 87% in predicting asthma severity.

Conclusions: Despite that spirometry and clinical classification are the gold standards for grading of asthma, Nasal lavage NFκB and cells can be considered as a new less-invasive non-subjective inflammatory marker for assessment of different grades of asthma severity and control.

Introduction

Bronchial asthma, a clinical complication of persistent inflammation of airways and subsequent airway hyperresponsiveness, is a leading cause of morbidity and mortality [1]. Despite that airway inflammation is a useful marker of

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disease activity, there is less information concerning airway inflammation in young children, in whom access to the lower airway is a major obstacle and in whom the asthma phenotype is thought to be more heterogeneous [2].

The upper airway, especially nasal epithelium, shares several properties with the lower airways. Moreover, it is easily accessible and nasal lavage (NAL) is well tolerated even by infants as young as 4 wk old. In comparison with serum biomarkers, measurements of NAL inflammatory markers can be assumed to reflect airway pathology more directly. Therefore, the determination of inflammatory markers in the nasal epithelium fluid would be attractive for both epidemiological and clinical purposes [3].

Nuclear factor kappa B (NF-kappa B) is a transcription factor that is critical for production of many inflammatory cytokines. It is activated in airway epithelium of both human asthmatics and mice after allergic stimulation [4]. It has been considered a master regulator of both innate and adaptive immune responses and it might play a cardinal role in allergic airway diseases [5]. Therefore, understanding various facets of regulation of NFκB and its targets may offer the potential to advance our knowledge of immune processes in asthma [6]. Moreover; no studies to date have identified its level in nasal lavage in correlation with asthma severity in children.

Aim of the work

This study aimed at evaluating diagnostic value of nasal lavage (NAL) nuclear factor kappa B and cytology, as a less-invasive bench-side maneuver, as inflammatory biomarkers in asthmatic children and to correlate it with asthma severity assessed clinically and by forced expiratory second 1 (FEV1) % of predicted for age and sex [7]; together with NAL cellularity as an indicator of airway inflammation.

Patients and methods

Study population and design

During the period from April, 2012 to October, 2014, this case–control study was carried out at the Pediatric Chest Clinic, Children's Hospital, Ain Shams University; Cairo, Egypt. It included 60 asthmatic children and 30-age and sex-matched- healthy children as a control group. Patients were selected according to the global initiative for asthma "GINA" [7]. An informed consent was obtained from parents of both patients and controls. The Pediatric Department Board ethically approved the study. None of the patients had a respiratory tract infection or exacerbation of asthma during enrollment. None of the asthmatics gave history for allergic rhinitis or previous nasal steroid intake (to assess local nasal epithelium as a representative for the lower airway inflammation). Neither asthmatics nor healthy children were exposed to second-hand tobacco smoking which may increase airway inflammation and NFκB level.

Asthmatic patients were further subdivided into allergic and non-allergic; based on atopic history and/or previous positive skin prick test [8]. Levels of asthma control were systematically assessed using GINA guidelines [7] during past three months: controlled versus partially/uncontrolled asthma. Severe exacerbations, according to ATS/ERS definition [9] and the number of days with symptoms (GINA guidelines) were specifically recorded.

Both patients and controls were examined, and then underwent spirometry and venous blood samples were collected to determine complete blood count for eosinophilia and nasal lavage fluid sampling for cytology and measurement of NFκB level.

Spirometry

A dynamic spirometry (Jaeger, Germany) was done for measurement of FEV1% of predicted; according to standards of the European Respirator Society and the American Thoracic Academy [10]. We used the highest values of FEV1 of three forced expiratory maneuvers.

Nasal lavage sampling

We clearly explained the maneuver for the child and parents as described [3]. Older children ($\geqslant$7 y) sat on a chair, with their head bent backward; they were instructed to hold their breath and to make a hiccup sound thus to close soft palate and prevent fluid being swallowed. We instilled 5 ml of pre-warmed isotonic using a needleless syringe. Then the child was asked to hold his/her breath for 10 s and then expel the fluid into a specimen cup. For younger children, lavages were performed in a supine position. Pre-warmed (2 ml) isotonic saline was instilled into each nostril and immediately aspirated into a specimen trap by inserting a flexible suction catheter. After washing each nostril, 1 ml of saline was aspirated through the catheter to rinse secretions into the trap. A proteinase inhibitor "phosphoramidon" was added to the specimen cup before collection of the expelled fluid [11–13].

NAL cytology

For analysis of cellular components, samples were processed quickly. A mucolytic agent; dithioerythritol (Sputalysin) was added to the NAL sample. After centrifugation, total cell count was obtained by a manual hemocytometer and differential cell counts by preparation of cytocentrifuge slides. Specimens were stained using the May-Grünwald-Giemsa method. Eosinophil, macrophage, lymphocyte and neutrophil counts are then expressed as cell count $\times 10^4$/ml [12–14]. Aliquots of the supernatant were stored at 70 °C for later analysis of NFκB.

Nasal lavage fluid NFκB measurement

Nuclear protein was isolated from the thawed cell pellet in a three step process according to the manufacturer's instructions (Nuclear Extract Kit; Cayman chemical company, Ann Arbor, MI, USA). The protein concentration was determined by Coomassie protein assay. NFκB in nuclear protein extract was determined by enzyme linked immunosorbent assay (Cayman chemical company). Assay specificity was confirmed by using the provided competitive oligonucleotides. The provided HeLa cell extract was used to produce a standard curve. Results were expressed as concentration of NFκB μg/μg nuclear protein.