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Original article

Sputum epithelial cell-derived neutrophil-activating peptide-78 (ENA-78/CXCL5) in asthmatic children: relation to eosinophil activation

Background: *Epithelial cell-derived neutrophil-activating peptide-78 (ENA-78) is a chemokine that recruits and activates neutrophils, possesses angiogenic properties and promotes connective tissue remodeling. Thus, it could play a pathogenic role in allergic airway inflammation. Eosinophils are the major source for this chemokine in inflamed airways.*
Objective: *To evaluate sputum ENA-78 expression and its relation to acute asthma exacerbations of varying severity, and eosinophil cationic protein (ECP) as a marker of eosinophil activation, as well as eosinophil counts in blood and sputum.*
Methods: *Sputum ENA-78 and serum ECP were measured by ELISA in 21 children during and after acute asthma exacerbation and 21 healthy matched controls. Patients were subdivided according to exacerbation severity into three equal subgroups; mild, moderate and severe.*
Results: *Sputum ENA-78 was significantly higher in asthmatic children during acute exacerbation than controls (310.1±156.9 pg/ml vs 65.9±11.6 pg/ml, p<0.0001). It was significantly higher in severe than moderate and in moderate than mild exacerbations, and was negatively correlated to the peak expiratory flow rate. Sputum ENA-78 showed significant positive correlations with serum ECP and eosinophil counts in blood and sputum. By follow up of patients with acute asthma exacerbation till remission of symptoms and signs, sputum ENA-78, serum ECP and eosinophil counts in blood and sputum decreased significantly, but their levels remained significantly higher than the control values.*
Conclusion: *Sputum ENA-78 is increased during acute asthma exacerbation and it positively correlates with exacerbation severity and eosinophil activation. Thus, it may play a role in the evolution of acute asthma exacerbation and may be a future target for new asthma therapeutic modalities.*

Keywords: Bronchial asthma; chemokines; children; epithelial cell-derived neutrophil-activating peptide-78; eosinophils; eosinophil cationic protein; sputum markers.

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INTRODUCTION

Airway inflammation is a characteristic feature of bronchial asthma. It contributes significantly to many features of this disease, including airflow obstruction, bronchial hyperresponsiveness, and the initiation of the injury-repair process (remodeling) found in some patients[1]. Assessing airway inflammation is important for investigating the mechanism and following the progression and resolution of the disease. This is difficult to be detected clinically, and may result in delays in initiating appropriate therapy. Thus, assessing biological markers of airway inflammation for proper diagnosis, monitoring and treatment of bronchial asthma is essential[2].

Sputum induction is a non-invasive test that monitors airway inflammation. It samples the lower airways more directly, it is considered a good alternative to fibro-optic bronchoscopy and it correlates well with data from bronchial biopsies and bronchoalveolar lavage[3]. Sputum markers tend to be more sensitive than blood tests when assessing airway inflammation[4].

Chemokines are a large group of chemotactic cytokines. They have been divided into four groups, designated CXC, CC, C and CX3C, depending on the spacing of conserved cytokines (where X is an amino acid). The CXC chemokines mainly target neutrophils and lymphocytes whereas the CC chemokines target a variety of cell types, including macrophages, eosinophils, basophils and dendritic

cells[5]. Recruitment of inflammatory cells is a hall phenomenon of all inflammatory diseases, including allergic asthma. Not only are chemokines involved in recruitment of these cells, but they also play a role in their activation and differentiation[6].

Eosinophils are the chief effector inflammatory cells contributing to the pathogenesis of bronchial asthma. Activated eosinophils in asthmatic patients release their granular proteins. One such protein is eosinophil cationic protein (ECP) that has an essential role in the pathogenesis of airway hyperresponsiveness due to its cytotoxic effect on airway epithelial cells[7].

Epithelial cell-derived neutrophil-activating peptide-78 (ENA-78/CXCL5), is a CXC chemokine that attracts neutrophils[8]. In the healthy lung, epithelial cells are the primary source of chemokines, while in the inflamed lung infiltrating cells within the submucosa are the major cellular source of chemokines[9]. Immunocytochemistry detected ENA-78 in eosinophils and the peptide was localized in the specific granules. This may suggest that, through expression of ENA-78, eosinophils can recruit and activate CXC receptor 2 (CXCR2)-bearing cells such as neutrophils at sites of inflammation. Eosinophils may also promote connective tissue remodeling through release of this peptide. Thus, eosinophils are not just mere targets for chemokines, but are also able to elaborate these factors in a manner that suggests an important immunoregulatory role[10].

Chemokines and their receptors are important potential therapeutic targets in allergy and asthma because of their central role in cell recruitment and activation during inflammation[6]. Targeting the chemokine system is generally done by affecting chemokine receptor binding which can be done in three ways: by using blocking antibodies, by modification of chemokines and therefore antagonizing chemokine receptors and by using small molecule chemokine receptor antagonists[11].

This study was conducted to measure sputum ENA-78 in relation to acute asthma exacerbation of varying severity, ECP, as a marker of eosinophil activation, and eosinophil counts in blood and sputum.

METHODS

Study population

This case-control, follow-up study was conducted on 21 asthmatic children recruited from the Pediatric Allergy and Immunology Clinic, Children's Hospital, Ain Shams University, over a period of 9 months. They were 14 (66.7%) males and 7 (33.3%) females, their ages ranged between 6 and 16 years with a mean age of 9.7 ± 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 was based on the clinical symptoms and signs of episodic wheezing, chest tightness, dyspnea and nocturnal symptoms that improved at least partially after bronchodilator therapy. Patients were studied within 24 hours of the start of an acute asthma exacerbation. The severity of acute asthma exacerbations was classified by symptoms (breathlessness, talking and alertness), signs (respiratory rate, use of accessory muscles, wheezes, pulse/min and pulses paradoxus) and functional assessment (peak expiratory flow rate "PEFR") following the National Heart, Lung and Blood institute, expert panel report 2, 2002[12]. Patients were excluded form the study if they had respiratory infection ruled out by the absence of clinical manifestations and chest radiograph findings.

Patients with acute asthma exacerbation were followed up for 2-3 weeks until stabilization of their condition and quiescence of asthma symptoms by treatment (i.e. remission or steady state). Then, follow-up blood and sputum samples were collected.

Twenty-one healthy children without a history of asthma or other atopic conditions and without family history of atopy were studied as controls. They were 14 (66.7%) males and 7 (33.3%) females, their ages ranged between 6 and 16 years with a mean age of 9.8 ± 3.8 years.

An informed consent was obtained form the parents or caregivers of each child before enrollment in the study.

Study measurements

Measurement of peak expiratory flow rate: Mini-Wright Peak Flow Meter (Clement Clarke International Ltd, Harlow, England) was used. Since the parents did not know their children's personal best, the estimation of the expected PEFR was obtained from a standardized chart that approximates PEFR by the child's height[13]. The PEFR of each patient was compared with the normal population and % predicted was calculated.

Assessment of complete blood count: One mL of venous blood was collected into sterile EDTA tubes and assayed by Coulter counter on the same day (Coulter Microdiff 18, Coulter Corp, Miami FL, USA). The differential leucocytic counts were counted manually from the blood film and expressed in absolute count values. The patient was

considered to have elevated blood eosinophil count if it was above 400 cells /mm^3.

Immunoglobulin E assay: serum total IgE was assayed by ELISA (Eurogenetics, IgE Quantitative, Tessen Derlo, Belgium). Results were expressed in IU/mL. Owing to the variability in serum total IgE levels with age in childhood, we calculated the percentage values from the reference ranges[14] by dividing the subject's level by the highest normal for age x 100.

Assessment of serum ECP: In the assay, the solid phase "a polysterene bead enclosed within an immulite test unit" is coated with a monoclonal antibody reactive to ECP, while the patient sample and alkaline phosphatase conjugated poloyclonal antibody are incubated for 30 min. at 37^{o}C. ECP in the sample is bound to form an antibody sandwich complex. Unbound conjugated antibody is removed by a centrifugal wash after which the substrate (adamantly dioxetane phosphate) is added and the test unit is incubated for further 10 minutes. Dephosphorylation of this substrate would result into production of an unstable union intermediate. Continuous production of this intermediate, resulted in sustained emission of light which is directly proportional to ECP in the sample[15]. As the data distribution was non-parametric, a patient was considered to have elevated serum ECP if it was above 18 ng/ml which was above the 95th percentile of the control values.

Sputum induction: Subjects under study were selected above 5 years of age so that adequate sputum samples can be obtained spontaneously when required. Sputum was induced only when it could not be produced spontaneously. The sputum induction was performed as a modification of the method described by Fahy et al.[16]. All subjects were premedicated with two puffs inhaled sulbutamol (200 μg) 30 minutes before sputum induction. To minimize contamination with saliva and postnasal drip, subjects were asked to rinse their mouths and blow their noses before induction and, wherever possible, before expectoration. Subjects inhaled 3% hypertonic saline solution aerosols generated by Farmasol compressor nebulizer (CA-MI s.n.c., Pilastro PR, Italy). Hypertonic saline was inhaled for 10-15 minutes according to the severity of asthma until an adequate volume of sputum was expectorated. Patients were asked to cough deeply and frequently during hypertonic saline inhalation. They were asked to cough the sputum into a sterile plastic container. The sample volumes and duration of sputum induction were recorded. Sputum induction was stopped if the PEFR% predicted fell by $\geq$ 15%.

Measurement of sputum eosinophils: sputum was selected form saliva and processed within 2 h. The method of sputum examination described by Popov et al.[17] was modified[18]. Sputum obtained was treated by adding equal volumes of 0.1% of dithiothreitol (ICN Biomedicals, Inc., Irvine, CA, USA), followed by equal volumes of Dulbecco's phosphate buffered saline (D-PBS). The sample was then mixed gently and placed in shaking water bath at 37^{o}C for 15 min to ensure complete homogenization. The sample was removed form the water bath periodically for further brief gentle mixing. Then, centrifugation was carried out at 1500 x g for 15 min. The cell pellet was spread on a slide that was stained with Leishman and a differential cell count of 400 non squamous cells was performed. Eosinophils in sputum are expressed as percentage of leucocytes. Evaluation was carried out by a single person, blind to the clinical conditions of the patients. As data distribution was non-parametric, patients were considered to have elevated sputum eosinophils % if it was above 2% (the 95th percentile of the control values).

Assessment of sputum ENA-78: Sputum samples were filtered through a two layer sterile gauze into sterile plastic vials (falcon, Oxnard, CA), centrifuged at 4^{o}C and 500g for 10 minutes. The supernatant was removed and stored at -70^{o}C until assay of ENA-78. This assay employs the quantitative sandwich enzyme immunoassay technique (R and D systems Inc., 614 Mclinley Place NE Minenopolis MN 55413, USA). A monoclonal antibody specific for ENA-78 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ENA-78 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for ENA-78 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ENA-78 bound in the initial step. The color development is stopped and the intensity of the color is measured by ELISA recorder[19]. The patient was considered to have an elevated sputum ENA-78 level if it was above the chosen highest cut-off value "82.8 pg/ml" which was the 95th percentile of control values because data distribution was non-parametric.

Statistical analysis:

The results were analyzed by commercially available software package (Stat View, Abacus Concepts, Inc, Berkley, CA, USA). The data were

presented as mean and standard deviation (SD) in addition to median and interquartile range (IQR) which is the difference between the 75th and 25th percentiles. Mann Whitney test was used for comparison between 2 groups as data distribution was non-parametric. Wilcoxon signed rank test was used for comparison between the same groups during and after exacerbation. Spearman's correlation coefficient "r" was used to determine the relationship between different quantitative variables. For all tests, a probability (p) of less than 0.05 was considered significant.

RESULTS

Sputum ENA-78 in relation to acute asthma exacerbation of varying severity:
Children with acute asthma exacerbation had significantly higher levels of sputum ENA-78 than healthy controls. When patients with acute asthma exacerbation were followed-up till remission, sputum ENA-78 levels decreased significantly but its levels remained significantly higher than healthy controls (table 1). In addition, sputum ENA-78 levels were significantly higher in severe than mild and moderate and in moderate than mild exacerbations (table 2).

Sputum ENA-78 in relation to steroid therapy
Sputum ENA-78 levels were significantly higher in patients who were receiving inhalation and/or systemic steroid therapy (n = 8; 2 had moderate and 6 had severe exacerbations) than in those who were not (n = 13; 7 had mild, 5 had moderate and one had severe exacerbations). Figure 1.

Serum eosinophil cationic protein and eosinophil counts in blood and sputum in relation to acute asthma exacerbation of varying severity:
Patients with acute asthma exacerbation had significantly higher levels of serum ECP, blood eosinophil counts and sputum eosinophils % than healthy controls. When these patients were reexamined during remission, the levels of these inflammatory markers decreased significantly in spite of still being significantly higher than controls. Serum ECP levels were significantly higher in severe than mild and moderate and in moderate than mild exacerbation. In contrast, blood eosinophil counts did not differ significantly between patients with mild, moderate and severe exacerbation. On the other hand, sputum eosinophils % were significantly higher in severe than mild and moderate exacerbation, but their levels in patients with mild and moderate exacerbation were comparable (table 3).

Table 1. Comparison of sputum levels of ENA-78 between asthmatic patients and controls.

	Sputum ENA-78 (pg/ml)		**All patients during exacerbation vs controls z (p)**	**All patients during remission vs controls z (p)**	**All patients during exacerbation vs remission z (p)**
	Mean ±SD	**Median (IQR)**			
All patients during exacerbation (n=21)	310.1 ±156.9	260 (260-5)	5.32 (<0.0001)**	4.4 (<0.0001)**	3.98 (<0.0001)**
All patients during remission (n=21)	174.1 ±114.9	129 (192.5)			
Controls (n=21)	65.9 ±11.6	66 (20)			

ENA-78: epithelial cell-derived neutrophil-activating peptide-78, p < 0.0001**: highly significant.

Table 2. Sputum ENA-78 in relation to severity of asthma exacerbation.

	Sputum ENA-78 (pg/ml)		Mild vs moderate z (p)	Mild vs severe z (p)	Moderate vs severe z (p)
	Mean ±SD	Median (IQR)			
Patients with mild exacer. (n=7)	165.6 ±42.3	180 (4)			
Patients with moderate exacer. (n=7)	270.9 ±41.8	296 (70)	3.1 (<0.01)**	3.1 (<0.01)**	2.6 (<0.01)**
Patients with severe exacer. (n=7)	494 ±126.6	562 (202)			

ENA-78: epithelial cell-derived neutrophil-activating peptide-78, $p < 0.01$: highly significant.

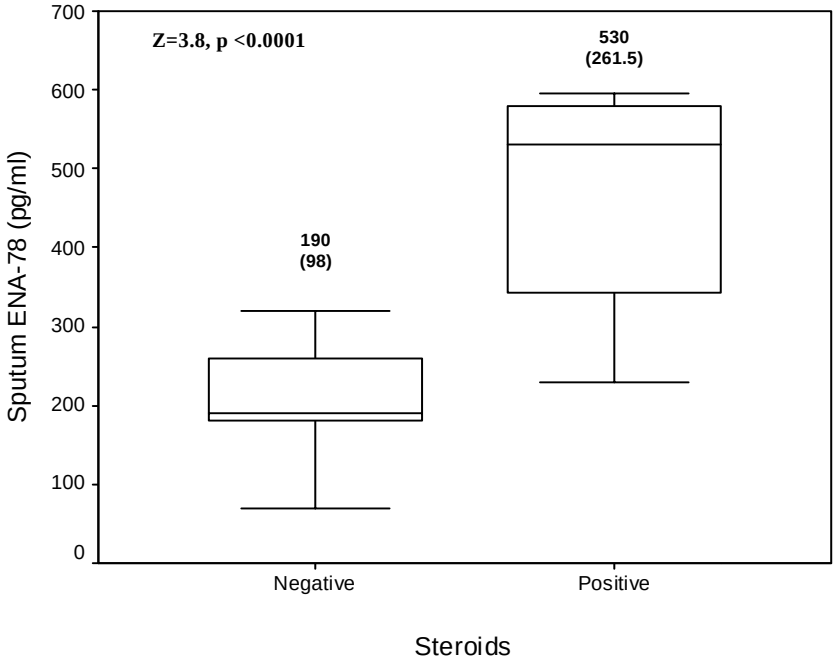

Figure 1. Sputum ENA-78 levels in relation to steroid therapy.
The boxes enclose the interquartile ranges (IQR) which are between the 25th and 75th percentiles. The horizontal line inside the box represents the median and the whiskers represent the non outlier or extreme maximum and minimum values.

Percentage of patients with elevated levels of the studied inflammatory markers for acute asthma exacerbation:

In asthmatic children, whether compiled in one group or subdivided into mild, moderate and severe according to exacerbation severity, sputum ENA-78 recorded the highest percentage of elevation among patients followed by serum ECP and sputum eosinophils %. The least percentage of elevation among patients was of blood eosinophil counts (table 4).

Correlation between sputum ENA-78 and the other studied inflammatory markers of bronchial asthma during exacerbation:

Sputum ENA-78 had significant negative correlation with PEFR. On the other hand, sputum ENA-78 had significant positive correlation with serum ECP, blood eosinophil count, sputum eosinophils % (figure 2) and serum IgE ($r = 0.5$, $p< 0.05$).

In contrast, sputum ENA-78 did not correlate significantly with age of patients and duration of illness ($p>0.05$).

Table 3. Serum ECP and eosinophil counts in blood and sputum in the different groups of the study.

	Serum ECP (ng/ml)		**Blood eosinophil counts (cells/mm^3)**		**Sputum eosinophils (%)**	
	Mean ±SD	**Median (IQR)**	**Mean ±SD**	**Median (IQR)**	**Mean ±SD**	**Median (IQR)**
All patients during exacerbation (n=21)	61.7 ±39.4	64 (69)	910.5 ±809	420 (1160)	4.9 ±4.1	3 (7)
All patients during remission (n=21)	28 ±19.5	18 (17.5)	577.7 ±697.9	185 (1003)	3 ±3	2 (5)
Controls (n=7)	15.2 ±2.5	16 (3)	96.8 ±67.8	90 (96.5)	0.6 ±0.7	1 (1)
Patients with mild exacer. (n=7)	25.6 ±6.5	28 (13)	396.4 ±185.6	390 (50)	2.6 ±1.5	3 (1)
Patients with moderate exacer. (n=7)	60.9 ±21.2	68 (10)	638.7 ±301.4	790 (440)	3 ±2.5	3 (2)
Patients with severe exacer. (n=7)	98.7 ±39.3	110 (42)	1696 ±970	2210 (1980)	9.1 ±4	11 (5)
z1 (p)	4.5 (< 0.0001)**		5.3 (<0.0001)**		4.5 (<0.0001)**	
z2 (p)	2.5 (<0.05)*		3.2 (<0.01)**		3.1 (<0.01)**	
z3 (p)	3.98 (<0.0001)**		3.22 (<0.01)**		3.87 (<0.0001)**	
z4 (p)	2.2 (<0.05)*		1.28 (>0.05)		0.27 (>0.05)	
z5 (p)	2.3 (<0.05)		1.6 (>0.05)		2.4 (<0.05)*	
z6 (p)	2.4 (<0.05)*		1.6 (>0.05)		2.3 (<0.05)*	

ECP: eosinophil cationic protein, p>0.05: non significant, p<0.05*: significant, p<0.01**, 0.0001**: highly significant, z1: comparison between asthmatic patients during exacerbation and controls, z2: comparison between asthmatic patients during remission and controls, z3: comparison between asthmatic patients during and after exacerbation, z4: comparison between patients with mild and moderate exacerbation z5: comparison between patients with mild and severe exacerbation, z6: comparison between patients with moderate and severe exacerbation.

Table 4. Percentage of patients with elevated levels of the studied inflammatory markers for acute asthma exacerbation.

Asthma inflammatory marker	**All asthmatic patients (n = 21)**	**Patients with mild exacerbation (n = 7)**	**Patients with moderate exacerbation (n = 7)**	**Patients with severe exacerbation (n = 7)**
Sputum ENA-78	95.2%	85.7%	100%	100%
Serum ECP	80.9%	71.4%	85.7%	85.7%
Sputum eosinophils%	71.4%	57.1%	71.4%	85.7%
Blood eosinophil count	57.1%	43%	57.1%	71.4%

ENA-78 = epithelial cell–derived neurotrophil activating peptide-78, ECP: eosinophil cationic protein.

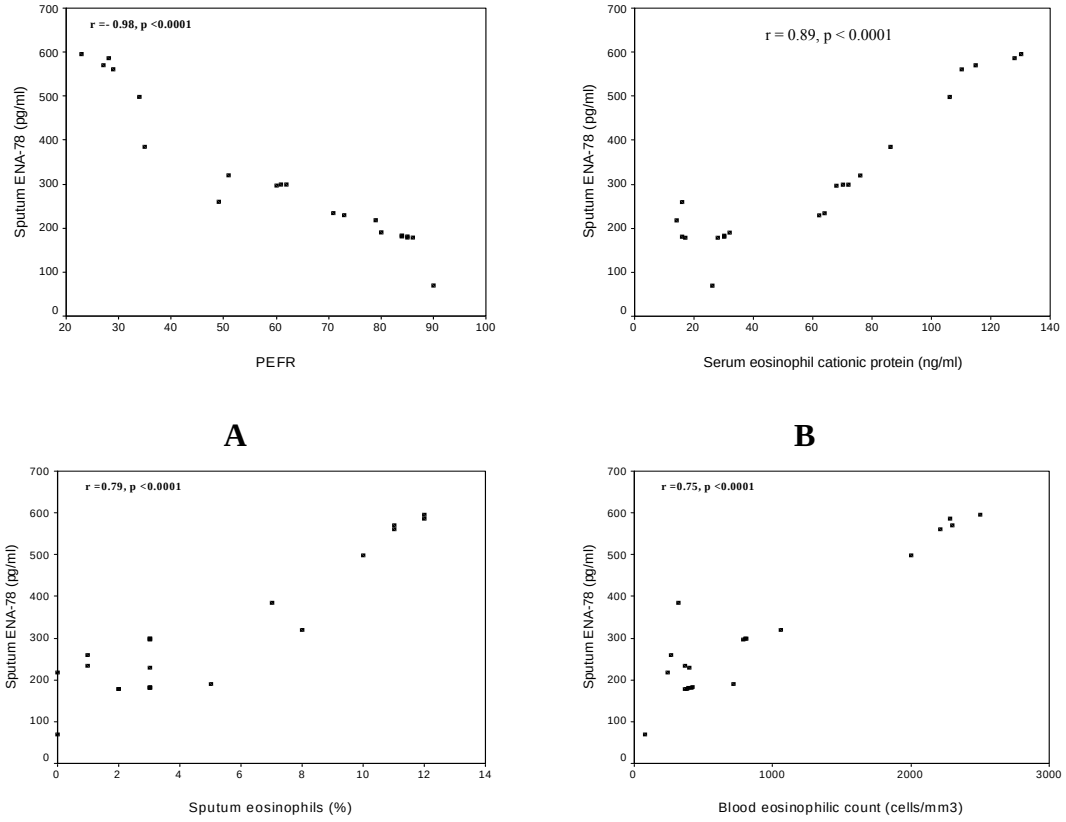

Figure 2. Negative correlation between sputum ENA-78 and PEFR (Fig. 2A) and positive correlation between sputum ENA-78 and serum ECP (Fig. 2B), sputum eosinophils % (Fig. 2C) and blood eosinophil count (Fig. 2D).

DISCUSSION

Epithelial cell-derived neutrophil-activating peptide-78 is an inflammatory C-X-C chemokine that is encoded by the CXCL5 gene[20]. Its levels were elevated in myriad inflammatory conditions[21,22] including pulmonary disease[23]. In our series, sputum ENA-78 was significantly higher in asthmatic patients during exacerbation than healthy controls. A previous study investigated the relationship between ENA-78 and rhinovirus (RV) infection-induced bronchospasm as asthma exacerbations are frequently associated with RV infection. Nasal ENA-78 levels were significantly elevated in asthmatic patients with than those without RV infection as RV16 induced 3-fold increases in ENA-78 gene transcription. Thus, ENA-78 may be produced by bronchial epithelial cells in response to RV16 infection resulting in initiation of neutrophil airway inflammation and development of virus associated airway pathologies as bronchospasm[24].

ENA-78 is an α chemokine which is produced concomitantly with IL-8 and melanoma growth stimulating activity[8]. The main stimuli for secretion of chemokines including ENA-78, are the early signals elicited during innate immune response. For example, bacterial products, viral infection and pro-inflammatory cytokines[25]. Notably, chemokines are induced rapidly, within one hour, by these triggers and provide an important link between early innate immune responses and adaptive immunity[9]. ENA-78 can act in concert with IL-8 to stimulate neutrophil directed chemotaxis, neutrophil activation via increasing the intracellular level of free calcium and elastase release and it can also induce neutrophil proadhesive activity[21,22]. Neutrophils contribute to the development of allergic asthma as they play a role in tissue remodeling. This ability stems in part from the angiogenic property of a subgroup of neutorphil activating CXC chemokines including ENA-78[26].

In our series, sputum ENA-78 levels measured during exacerbation had significant positive correlation with serum total IgE levels. Such finding added to the increasing evidence that this chemokine may be implicated in asthma

pathophysiology as IgE is closely related to the pathogenesis of asthma. However, a good number of atopic asthmatics have normal total serum IgE levels[13].

Eosinophilia measured both in the peripheral circulation and in the airways is a characteristic feature of asthma[26]. This was also the case in our study as asthmatic patients had significantly higher blood and sputum eosinophil counts than controls. Similar findings were reported by other investigators[27,28]. In addition, activation and degranulation of eosinophils, the chief effector inflammatory cells in bronchial asthma, release a wide variety of tissue damaging products capable of worsening this condition[26]. One such product is ECP which has an essential role in the pathogenesis of airway hyperresponsivneess due to its cytotoxic effect on airway epithelial cells[7]. In the present work, children with acute asthma exacerbation had significantly higher serum ECP than healthy controls. Other investigators also reported elevated serum ECP during acute asthma exacerbation[29].

In our study, sputum ENA-78 had significant positive correlation with ECP and eosinophil counts in blood and sputum. Some investigators reported that eosinophils express enhanced levels of ENA-78 in their specific granules detected by immuno-electron microscopy suggesting that eosinophils are capable of recruiting and activating CXCR2 bearing cells, such as neutrophils through the release of this chemokine[10].

Treatment applications in asthma are based upon disease severity. Since asthma is a chronic disease whose severity may change over time, it is important to adjust treatment to appropriately match treatment requirements with disease severity. Failure to do this leads to under-treatment with the associated risk of impaired quality of life and severe exacerbations. Alternatively over-treatment may occur, with the risk of excessive adverse effects. At present, symptoms and lung functions are used to monitor asthma severity and adjust treatment. These measures are imperfectly correlated with the underlying pathophysiological process in asthma, namely airway hyperresponsivenss and airway inflammation. Using objective measures of airway hyper-responsiveness and airway inflammation may lead to better management of asthma[30].

In the present work, sputum ENA-78 correlated positively with the disease severity as evidenced by the graded increase in its levels that went hand in hand with the degree of severity of asthma exacerbation. Another finding which supported this argument was the significant negative correlation between sputum ENA-78 and PEFR. Thus, the relationship between ENA-78 and bronchial asthma may be a causal one in which this chemokine may not only play a role in the evolution of acute asthma exacerbation, but its levels may also determine its clinical severity. We could not trace data in the literature that address the relationship between ENA-78 and the severity of acute asthma exacerbation. Thus, broad scale studies are warranted to set cut-off values of ENA-78 for each grade of asthma severity. Indeed, this will allow for a better management of asthma exacerbations.

In contrast to sputum ENA-78, blood eosinophil count did not correlate significantly with the severity of acute asthma exacerbation. Also, sputum eosinophils % did not accurately reflect the severity of asthma exacerbation as there were no significant differences between mild and moderate exacerbations. Some investigators reported that eosinophil cell numbers do not necessarily equate with function as hypodense eosinophils (eosinophils with lower density, and greater number of immunoglobulin receptors) better reflect the severity of asthma than the mere count of peripheral blood eosinophils. These hypodense cells are metabolically more active and proinflammatory than its normal dense counter parts and they are important in the cascade of events which often determines asthma severity[31]. Thus, the significant positive correlation between sputum ENA-78 and asthma severity, serum ECP, eosinophil counts in blood and sputum and serum IgE may denote that this chemokine may reflect the inflammatory process in bronchial asthma even in patients with mild exacerbations in the meantime when eosinophil counts in blood and sputum were not elevated in a large proportion of these patients.

Corticosteroid-treated asthmatic patients had significantly higher levels of sputum ENA-78 than non-steroid treated patients. This was surprising because corticosteroids were supposed to control the inflammation of the airways with subsequent decrease of the levels of the inflammatory indices. This could be explained by the fact that corticosteroids were used mostly by inhalation in our patients categorized to have severe asthma exacerbations. In spite of steroid therapy, acute severe exacerbations may result from failure of many children to introduce the medication into their lung especially in absence of spacer or air chamber delivery systems.

In the present work, although sputum ENA-78, serum ECP and eosinophil counts in blood and sputum were significantly lowered during asthma quiescence, yet their levels remained significantly

higher than those of controls. Other investigators reported lower eosinophil number in blood and eosinophil proteins levels in serum (ECP and eosinophil peroxidase) in healthy children than in the symptom – free asthmatic children[32]. These findings may denote that in spite of apparent clinical quiescence of bronchial asthma, there is underlying inflammation with release of chemokines such as ENA-78 from eosinophils. This undermines the importance of clinical examination alone as a guide to the subsidence of asthma exacerbation. This also highlights the importance of searching for inflammatory markers of bronchial asthma such as ENA-78 for the objective measurement of asthma quiescence.

In conclusion, sputum ENA-78 is increased during acute asthma exacerbations, and its levels closely follows its severity, and positively correlates with markers of eosinophil activation. Thus, ENA-78 possibly plays a role in the evolution of acute asthma exacerbation and hence, it may be a target for new therapy of asthma exacerbations.

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