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Original article

Brain-derived neurotrophic factor in asthmatic children.

Background: *Brain-derived neurotrophic factor (BDNF) regulates the cross-talk between the immune and nervous systems which may play an important role in asthma pathophysiology.*

Objective: *This study was aimed to investigate the relation between BDNF and asthma exacerbation and severity, and to study its possible correlation to eosinophilic counts in blood and sputum.*

Methods: *Twenty-seven asthmatic children were studied during both exacerbation and remission. According to acute exacerbation severity as assessed clinically and by peak expiratory flow rate (PEFR), they were equally subdivided into 3 groups (mild, moderate and severe). Serum and sputum BDNF levels as well as blood and sputum eosinophilic counts were estimated in all patients in comparison to 30 healthy children with no personal or family history of atopy.*

Results: *BDNF levels (in serum and sputum) and eosinophilic counts (in blood and sputum) were significantly elevated in asthmatic patients, whether studied as one group or subgrouped into mild, moderate and severe as compared to controls. Patients with mild, moderate and severe acute asthma exacerbation had significantly higher values of BDNF (in serum and sputum) and eosinophilic count (in blood and sputum) than the corresponding values measured during remission. The latter values were still higher than those of the control group. BDNF in serum and sputum indirectly correlated with asthma severity as evidenced by their negative correlation with PEFR. However, sputum BDNF correlated better with the severity of asthma exacerbation as evidenced directly by its significant increase with clinical severity. Both serum and sputum BDNF levels revealed significant positive correlations with eosinophilic count in blood and sputum among all studied groups.*

Conclusion: *BDNF probably plays a role in the evolution of asthma exacerbation and it reflects the degree of asthma severity during exacerbation. It might also represent an objective indicator of remission and treatment efficacy. Studies with specific BDNF receptor antagonists or synthesis inhibitors are required as BDNF may prove to be a reasonable target for a new therapy in future.*

Key words: BDNF, neurotrophins, bronchial asthma, asthma severity, neurogenic inflammation.

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INTRODUCTION

The development and maintenance of asthma is thought to involve the nervous system and the immune system[1]. The regulatory network between the immunological events and the neuronal control of airway smooth muscle contractility remains to be defined[2]. Neurotrophins represent candidate molecules regulating and controlling this cross-talk between the immune and nervous system[3]. Neurotrophins are a family of peptides that promote survival, growth and differentiation of neurons. They include; nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3)[4]. They are expressed by resident lung cells[3]. Their function in lungs is currently poorly understood. They may play an important role in the pathophysiology of airway hyperresponsiveness during allergic inflammation[2].

The main purposes in developing inflammatory indices for asthma are to help early detection and differential diagnosis of the disease and further to make possible better targeted treatment as well as follow up of patients on the basis of objective

measurements. The need for simple parameter to detect and monitor airway inflammation is obvious especially in general practice[5]. The aim of the present study was to investigate the contribution of neurotrophins, through measuring one of them (BDNF), in asthma exacerbation and severity and to study its possible correlation to eosinophils in blood and sputum.

METHODS

This study was conducted on 27 asthmatic children recruited from the Pediatric Allergy and Immunology Clinic, Children's Hospital, Ain Shams University. They were 18 (66.7%) males and 9 (33.3%) females, their ages ranged from 5 to 15 years with a mean age of 9.34 ± 3.57 years.

Thirty healthy children without history of asthma or other atopic conditions and without family history of atopy were studied as controls. They were 20 (66.7%) males and 10 (33.3%) females, their ages ranged from 5 to 15 years with a mean age of 9.6 ± 3.66.

The diagnosis of asthma was based on the clinical symptoms and signs of episodic wheezing, chest tightness and dyspnea that improved at least partially after bronchodilator therapy[6]. Patients were studied within 24 hours of the start of an acute asthma exacerbation (defined by increasing cough, wheezing, dyspnea and nocturnal symptoms) and during remission. The interval between acute asthma exacerbation and remission ranged between 3 and 8 days.

Patients were classified into 3 groups according to the severity of acute asthma exacerbation as follows:

Group I (patients with mild acute asthma exacerbation): It included 9 patients. They were 6 males and 3 females. Their ages ranged from 5 to 15 years with a mean age of 8.89 ± 3.72 years.

Group II (patients with moderate acute asthma exacerbation): It included 9 patients, they were 6 males and 3 females. Their ages ranged from 5 to 14 years with a mean age of 9.56 ± 3.54 years.

Group III (patients with severe acute asthma exacerbation): It included 9 patients. They were 6 males and 3 females. Their ages ranged from 6 to 15 years with a mean age of 9.55 ± 3.84 years.

All patients in this study were receiving quick-relief medications to relieve acute broncho-constriction as oral and or inhaled β_2 agonists. In addition, 8 patients were receiving inhaled corticosteroids for 1-4 years (2 had moderate acute exacerbation and 6 had severe acute exacerbation). Ten patients were also receiving sustained-release theophylline for 1-4 years (2 had moderate acute exacerbation and 8 had severe acute exacerbation).

Methods:

Patients were subjected to:

1. Clinical evaluation by history taking laying stress on duration of disease, frequency of acute attacks, nocturnal symptoms, predisposing factors, drug therapy and degree of clinical severity. Clinical chest examination was performed to verify asthma exacerbation and assess severity.
2. Classification of the severity of acute asthma exacerbation was made according to the protocol of the Pediatric Allergy and Immunology Unit, Ain Shams University[7]. In this classification, peak expiratory flow rate (PEFR) is included. Mini-Wright peak flow meter (Clement Clarke International Ltd., Edinburgh, Essex CM202DE, England) was used. The PEFR of each patient was compared with normal population and % predicted was calculated[8].
3. Classification of asthma severity during remission into mild intermittent, mild persistent, moderate persistent and severe persistent asthma according to National Heart, Lung and Blood Institute[9].
4. Complete blood count (CBC) to evaluate the number of eosinophils. The patient was considered to have elevated blood eosinophilic count[10] if it was above 400 cells/mm^3.
5. Serum total IgE by enzyme-linked immunoassay (ELISA)[11] (Pathozyme IgE, Omega Diagnostics Limited, UK).
6. Counting the number of eosinophils in sputum as percentage of leucocytes microscopically.
7. Measurement of serum and sputum brain-derived neurotrophic factor (BDNF) level by enzyme-linked immunosorbent assay (ELISA) method[12] (R and D systems, Oxon OX 14 3 YS, United Kingdom).

Measurements of PEFR, blood eosinophilic count, sputum eosinophils percentage and serum and sputum BDNF were repeated for all patients during remission.

Blood samples:

Five ml of venous blood were collected from each participated child. Two ml of blood were collected into sterile EDTA tubes for CBC assay by Coulter (Coulter JT 660, Coulter Electronics Ltd, UK) on the same day. The other 3 ml of venous blood were withdrawn from each patient and control and left to clot for 30 minutes at room temperature and the tube was centrifuged at 3000 rpm for 15 minutes. The separated serum was stored in sterile aliquots at -70°C till time of assay of serum IgE and BDNF.

Principle of the serum BDNF assay:
This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for BDNF had been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any BDNF present was bound by the immobilized antibody. An enzyme-linked monoclonal antibody specific for BDNF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of BDNF bound in the initial step. The color development was stopped and the intensity of the color was measured.

Sputum BDNF assay:
Sputum samples were filtered through a two layer sterile gauze into sterile plastic vials (Falcon, Oxnard, CA), centrifuged at 4°C and 500 x 9 for 10 minutes. The supernatant was removed and stored at -70°C until assay. Sputum obtained was treated by adding equal volumes of 0.1% of dithiothreitol (sputa-lysine 10%, Gibco BRL, Grandisland, New York, USA), followed by equal volumes of Dulbecco's phosphate buffered saline (D-PBS). The sample then mixed gently and placed in shaking water bath at 37°C for 15 minutes to ensure complete homogenization. The sample was removed from the water bath periodically for further brief gentle mixing. Then centrifugation was done at 1500 rpm for 10 minutes. The slide was stained with Leishman for differential leucocytic count. Eosinophils in sputum are expressed as percentage of total leucocytic count. A patient was considered to have elevated sputum eosinophils percentage if it was above 2% (95^{th} percentile of control values) as data distribution was non parametric.

Interpretation of BDNF results:
Patient was considered to have elevated serum or sputum BDNF level if it was above the highest cut off value (11.5 ng/ml for serum BDNF and 20 pg/ml for sputum BDNF). These values were located above the 95^{th} percentile of the control values as 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). Student's "t" test was used to compare parametric data, while Mann Whitney "U" test was used for non-parametric data. Pearson 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

Brain-derived neurotrophic factor levels (in serum and sputum) and eosinophilic count (in blood and sputum) were significantly elevated in asthmatic patients, either when they were studied as a whole or subgrouped into mild, moderate and severe, as compared to controls (table 1).

Effect of asthma exacerbation on the studied laboratory parameters:
Patients with mild, moderate and severe acute asthma exacerbation had significantly higher values of serum and sputum BDNF, blood eosinophilic count and sputum eosinophils% than the corresponding values during remission (table 1).

Asthmatic patients with severe acute exacerbation had significantly higher values of sputum BDNF, blood eosinophilic count and sputum eosinophils % than the corresponding values of patients with moderate and mild exacerbation. On the other hand, asthmatic patients with moderate acute exacerbation had significantly higher value of sputum BDNF than asthmatic patients with mild exacerbation. Although blood eosinophilic count and sputum eosinophils% in patients with moderate asthma exacerbation were higher than that of the patients with mild asthma exacerbation, yet, this difference was not statistically significant. In contrast to sputum BDNF levels which increased significantly with the disease severity, there were no significant differences in serum BDNF levels between patients with mild, moderate and severe acute asthma exacerbation (table 1).

Variation of the level of the studied asthma inflammatory indices in relation to steroid therapy:
Patients who were on steroids (systemic and/or inhalation therapy) during acute exacerbation had significantly higher values of BDNF (in serum and sputum), blood eosinophilic count and sputum eosinophils %, than the corresponding values of those who did not receive steroids (table 1).

Correlations of serum and sputum BDNF with other studied parameters:
Serum BDNF held significant positive correlations with blood eosinophilic count, sputum eosinophil percentage, sputum BDNF and serum IgE and

significant negative correlation with PEFR during asthma exacerbation among all studied groups of patients. On the other hand, sputum BDNF had significant positive correlations with blood eosinophilic count, sputum eosinophils% and significant negative correlation with PEFR during asthma exacerbation among all studied groups. It also had significant positive correlation with serum IgE among all asthmatic patients and patients with severe exacerbation only (table 2). On the other hand, neither serum nor sputum-BDNF showed significant correlations with age, height, weight, age of onset and duration of disease among all studied groups during exacerbation ($p>0.05$).

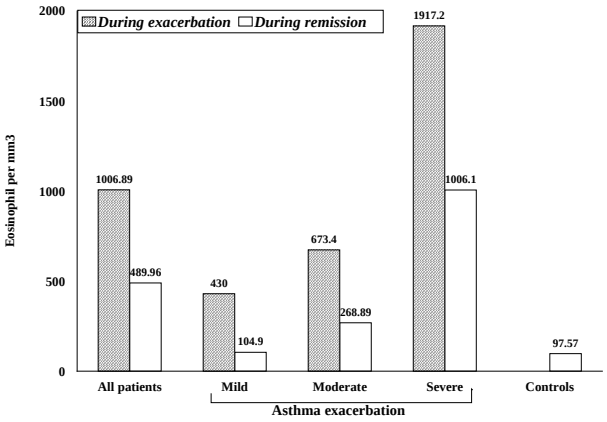

Figure (1): Mean levels of serum BDNF among all studied groups.

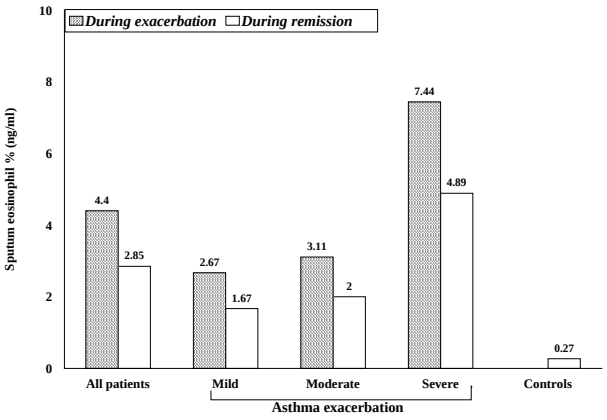

Figure (2): Mean levels of sputum BDNF among all studied groups.

Sputum BDNF had the highest diagnostic sensitivity (100%) followed by serum BDNF (92.6%), then blood eosinophilic count (74%) and the least sensitivity (55.5%) was of the sputum eosinophil percent for diagnosing acute asthma exacerbation. Similarly, sputum BDNF had higher sensitivity for diagnosing mild and moderate exacerbations (100% for both) than serum BDNF (88.9% for both), blood eosinophilic count (44.4% for mild exacerbation and 77.7% for moderate exacerbation) and sputum eosinophil percent (44.4% for mild exacerbation and 55.5% for moderate exacerbation). In severe exacerbation, the sensitivities of sputum and serum BDNF and blood eosinophilic count were equal (100%) and were higher than that of sputum eosinophil percent (66.7%). Sputum BDNF has much higher sensitivity for the differentiation of mild from moderate acute asthma exacerbations (100%), mild from severe exacerbations (100%) and moderate from severe exacerbation (88.9%) than did serum BDNF (11.1%, 22.2% and 11.1% respectively).

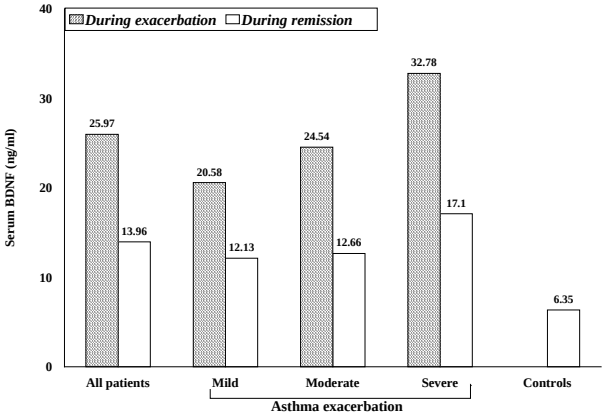

Figure (3): Mean levels of blood eosinophilic count among all studied groups.

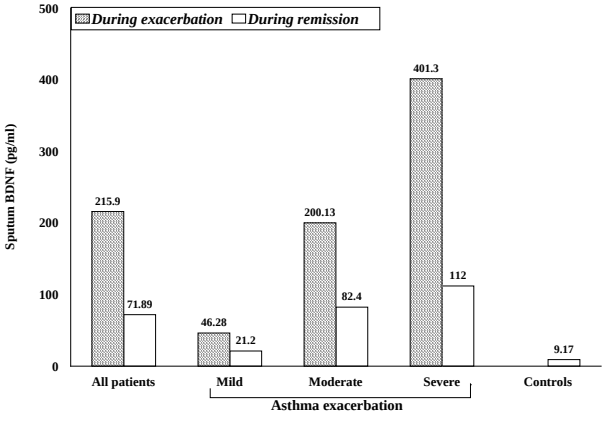

Figure (4): Mean levels of sputum eosinophils % among all studied groups.

Table (1): Variation of serum and sputum BDNF levels and blood and sputum eosinophil count with asthma activity and severity.

	Serum BDNF (ng/ml)		Sputum BDNF (pg/ml)		Blood eosinophilic count (cells/mm^3)		Sputum eosinophils (%)	
	Mean ± SD	**t/z (p)**	**Mean ± SD**	**t/z (p)**	**Mean ± SD**	**t/z (p)**	**Mean ± SD**	**t/z (p)**
Asthmatic patients during exacerbation vs controls	25.9±13.8 vs 6.4±2.4	2.38 (0.001)	215.9±162.5 vs 9.2±4.7	5.85 (<0.001)	1006.9±734.5 vs 97.6±64.5	6.2 (<0.001)	4.4± 3.9 vs 0.3± 0.6	4.84 (<0.001)
Asthmatic patients during remission vs controls	13.9±10.8 vs 6.4±2.4	4.62 (<0.001)	71.9±69.2 vs 9.2±4.7	5.9 (<0.001)	489.7±628.6 vs 97.6±64.5	3.6 (<0.001)	2.9± 2.7 vs 0.3± 0.6	5.07 (<0.001)
Mild exacerbation vs controls	20.6±6.0 vs 6.4±2.4	10.67 (<0.001)	46.3±15.7 vs 9.2±4.7	11.63 (<0.001)	430±198.9 vs 97.6±64.5	3.9 (<0.001)	2.7± 1.6 vs 0.3± 0.6	7.02 (<0.001)
Moderate exacerbation vs controls	24.5±9.7 vs 6.4±2.4	10.6 (<0.001)	200.1±59.2 vs 9.2±4.7	11.6 (<0.001)	673.4±303.6 vs 97.6±64.5	4.7 (<0.001)	3.1± 2.7 vs 0.3± 0.6	7.03 (<0.001)
Severe exacerbation vs controls	32.8±20.1 vs 6.4±2.4	9.6 (<0.001)	401.3±163.6 vs 9.2±4.7	18.04 (<0.001)	1917.2±435.4 vs 97.5±64.5	4.5 (<0.001)	4.4± 4.9 vs 0.3± 0.6	5.49 (<0.001)
Patients during and after mild exacerbation	20.6 ±6.0 vs 12.1± 4.1	7.52 (<0.001)	46.3±15.7 vs 21.2±8.9	4.19 (<0.001)	430±198.9 vs 104.9±75.9	6.98 (<0.001)	2.7± 1.6 vs 1.7± 1.2	6 (<0.001)
Patients during and after moderate exacerbation	24.6±9.7 vs 12.7±6.6	8.24 (<0.001)	200.1±59.2 vs 82.4±67.0	15.36 (<0.001)	673.4±303.6 vs 268.9±1917.2	3.1 (<0.001)	3.1± 2.7 vs 2± 2.1	4.62 (<0.001)
Patients during and after severe exacerbation	32.8±20.1 vs 17.1±17.4	10.05 (<0.001)	4011.3±103.6 vs 112±78.4	23.34 (<0.001)	1917.2±435.4 vs 1096.1±792.1	5.66 (<0.001)	7.4± 4.8 vs 4.9± 3.3	4.23 (<0.05)
Mild vs moderate exacerbation	20.6±6.0 vs 24.6±9.7	1.04 (> 0.05)	46.3±15.7 vs 200.1±59.2	7.53 (<0.001)	430±198.9 vs 673.4±303.6	2.01 (>0.05)	2.7± 1.6 vs 3.1± 2.7	0.43 (>0.05)
Mild vs patients with severe exacerbation	20.6±6.0 vs 32.8±20.1	1.74 (> 0.05)	46.3±15.7 vs 200.1±59.2	14.2 (<0.001)	430±198.9 vs	9.3 (<0.001)	2.7± 1.1 vs 7.4± 4.8	2.82 (<0.05)
Moderate versus patients with severe exacerbation	24.6±9.7 vs 32.8±20.2	1.17 (>0.05)	200.1±59.2 vs 200.1±59.2	5.05 (<0.001)	673.4±303.6 vs 1096.1±792.1	> 0.02 (<0.001)	3.1± 2.7 vs 7.1± 4.8	2.43 (<0.05)
Patients who received vs patients who did not receive steroids	35.7±20 vs 21.9±7.8	2.63 (<0.05)	392.5±139.9 vs 141.5±104	5.17 (<0.001)	1777.4±767.5 vs 682.5±418.5	4.82 (<0.001)	8.4± 4.3 vs 2.7± 2.2	4.6 (<0.001)

Table (2): Correlations between serum and sputum BDNF and other studied laboratory parameters during asthma exacerbation

Variable	Serum BDNF				Sputum BDNF			
	All patients	Mild asthma	Moderate asthma	Severe asthma	All patients	Mild asthma	Moderate asthma	Severe asthma
	r p	r p	r p	r p	r p	r p	r p	r p
PEFR	- 6.14 **<0.05**	0.99 **<0.05**	- 0.99 **<0.05**	- 0.75 **<0.05**	- 0.99 **<0.05**	- 0.78 **< 0.05**	- 0.99 **<0.05**	- 0.98 **<0.05**
Blood eosinophil	0.66 **<0.05**	1 **<0.05**	0.99 **<0.05**	0.77 **<0.05**	0.96 **<0.05**	0.76 **< 0.05**	1 **<0.05**	1 **<0.05**
Sputum eosinophils	0.76 **<0.05**	0.98 **<0.05**	0.93 **<0.05**	0.65 **<0.05**	0.85 **<0.05**	**< 0.05**	0.92 **<0.05**	0.97 **<0.05**
Sputum BDNF	0.65 **<0.05**	0.76 **<0.05**	0.99 **<0.05**	0.77 **<0.05**				
Serum IgE	0.75 **<0.05**	0.92 **<0.05**	0.56 **<0.05**	0.72 **<0.05**	0.83 **<0.05**	0.64 > 0.05	0.55 > 0.05	0.97 **<0.05**

Table (3): Sensitivity of the studied inflammatory indices for acute bronchial asthma exacerbation

Asthma inflammatory index	All asthmatic patients (n = 27)	Patients with mild exacerbation (n = 9)	Patients with moderate exacerbation (n = 9)	Patients with severe exacerbation (n = 9)
Serum BDNF	92.6%	88.9%	88.9%	100%
Sputum BDNF	100%	100%	100%	100%
Blood eosinophilic count	74.1%	44.4%	77.7%	100%
Sputum eosinophils%	55.5%	44.4%	55.5%	66.7%

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + false negative}} \times 100$$

True positive = Patients with acute asthma exacerbation and elevated marker level.
False negative = Patients with acute asthma exacerbation and normal marker level.

DISCUSSION

Both serum and sputum BDNF were significantly higher in asthmatic patients than controls during acute exacerbation, either when they were studied as a whole or subgrouped as mild, moderate and severe. Similar findings regarding serum BDNF were reported by Virchow et al.[12] who suggested that BDNF participates in the pathophysiology of allergic diseases including asthma.

To date, no available data regarding sputum BDNF levels in asthmatic children have been reported to be compared with our results. Virchow et al.[12] reported increased levels of neurotrophins (NGF, BDNF and NT-3) in the bronchoalveolar lavage fluid from patients with asthma after segmental allergen provocation meaning that neurotrophins are produced endobronchially following allergen provocation, suggesting their contribution to the pathogenesis of asthma. Investigations of the inflammatory response of airways to allergen challenge in asthma has usually relied on invasive techniques such as bronchial biopsy and bronchoalveolar lavage. The less invasive technique of sputum induction offers an alternative to invasive techniques[2].

Renz[3] mentioned that neurotrophins are elevated in asthmatic patients as they play an important role in the pathophysiology of asthma in several ways. The predominant effect on peripheral nerves innervating the lungs is described by the term “neuronal plasticity” which is defined as qualitative and / or quantitative changes in the functional activity and capacity of peripheral neurons leading to heightened autonomic reflex activity. These neurons control development of airway hyperresponsiveness and acute inflammatory responses resulting in the concept of “neurogenic inflammation” due to increased production of neuropeptides and tachykinins. Neurotrophins also exhibit profound effects on immune cells residing in airways and lung tissue. These effects are described by the term

"immunological plasticity"[13]. Thus, BDNF links pathologic events in bronchial asthma to dysfunctions of the immune and nervous system[3].

The traditional cellular sources of neurotrophins, including BDNF, under physiological conditions are primarily nerve-associated cells such as glial cells, Schwann cells or fibroblasts and neurons themselves[3]. Braun et al.[13] mentioned that in inflammatory processes, BDNF is also produced by a wide range of hematopoietic cells including mast cells, macrophages and T cells. In addition, airways epithelium constitutively expresses BDNF. Thus, BDNF may be an inflammatory product that links bronchial hyperreactivity to the inflammatory processes occurring in bronchial asthma. Wider-scale studies are needed to prove this argument.

BDNF liberation is important for the development of bronchial hyperresponsiveness in asthma by neurogenic inflammation. This pathway could be triggered by immune cells with the potential to release neurotrophins in allergic inflammation. Vice versa, immune cells are widely influenced by BDNF which is able to induce degranulation and differentiation of mast cells, the key cells of the early allergic response[14].

In our study, although sputum and serum BDNF correlated positively with the disease severity as evidenced by their significant negative correlation with PEFR, yet sputum BDNF correlated better with disease severity as evidenced by its significant elevation in patients with acute severe asthma exacerbation as compared to those with mild and moderate acute asthma exacerbations and also in patients with moderate acute asthma exacerbations as compared to those with mild acute asthma exacerbation. So, further studies are warranted to determine cut-off values for sputum BDNF which may aid in the classification of asthma severity.

Although serum and sputum BDNF levels decreased significantly during asthma quiescence, yet these levels were still significantly higher than that of controls. This finding denotes the underlying and ongoing neurogenic inflammation with release of neurotrophins such as BDNF. The latter may result in airway inflammation and hyperactivity that may be provocated easily at any time. This underscores the importance of clinical examination alone as a guide of subsidence and control of asthma exacerbation and highlights the importance of searching for inflammatory markers of bronchial asthma such as BDNF for objective measurement of asthma quiescence and guidance of proper response to asthma therapy.

The duration of illness in the present study did not influence serum and sputum BDNF levels neither did the age of the child nor the gender. The small sample size in the present study did not allow for proper analysis of epidemiological data.

Eosinophilia measured both in the peripheral blood and in the airway secretions is a characteristic feature of asthma. Furthermore the severity of the eosinophilia is correlated with objective markers of asthma severity such as lung function[15]. This was also the case in our series. The asthmatic patients had significantly higher blood eosinophilic counts and sputum eosinophils% than controls. Similar findings were reported by previous studies[16-18]. In our study, blood and sputum eosinophilic counts were significantly higher in patients with severe asthma exacerbation than those with mild and moderate exacerbations. Jang et al.[19] similarly reported increased sputum eosinophil counts in severe or life threatening asthma than in mild to moderate asthma. Although their levels in patients with moderate exacerbation seemed higher than those with mild exacerbation, yet this difference was not statistically significant. El-Gamal et al.[16] reported that there was a lack of significant difference in blood eosinophilic percentage between patients with moderate and those with severe asthma exacerbation. They suggested that priming of eosinophils occurs in all asthmatics irrespective of the severity of bronchospasm.

We found that both serum and sputum BDNF showed significant positive correlations with blood eosinophilic count in one hand and with sputum eosinophilic percent values in the other. This may suggest that BDNF has eosinophil chemotactic effect and this could be one of the ways by which it exerts its airway inflammatory effect, or alternatively it may represent one of the components of the late phase inflammatory response.

The high sensitivity of BDNF (both in serum and sputum) for asthma exacerbation together with its significant positive correlation with asthma severity and eosinophilic count in blood and sputum denote that this neurotrophin reflects the inflammatory processes in bronchial asthma even in mild exacerbations.

Corticosteroid-treated asthmatic patients had significantly higher eosinophilic count (in blood and sputum) and BDNF levels (in blood and sputum) than non-corticosteriod-receivers. This was surprising because corticosteroids were supposed to control inflammation of the airways with subsequent decrease in the levels of inflammatory indices. However, this could be explained by the

fact that corticosteroids were used mostly by inhalation in patients categorized to have moderate or severe persistent asthma whose symptoms are not well controlled. So, this finding might actually be a reflection of disease severity. Further wide-scale studies are warranted to investigate the variation in BDNF levels according to the mode of therapy in bronchial asthma.

In conclusion, BDNF plays a role in the evolution of asthma exacerbation and its levels (especially in the sputum) reflect the degree of the severity of asthma exacerbation and the ongoing inflammatory process during asthma quiescence. This highlights the importance of BDNF as an inflammatory marker detecting both the evolution and the control of asthma exacerbation. Therefore, it might represent an objective guide of treatment efficacy. Trials with specific BDNF receptor antagonists or synthesis inhibitors are required as BDNF may prove to be a reasonable target for a new therapy in the future. In addition, studies concerning the relation between BDNF and bronchial hyperreactivity are also required as it may be a link between hyperreactivity and the inflammatory process in bronchial asthma.

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