

## Determination the Role of Endothelial Cell-Specific Molecule-1 (ESM-1) in Childhood Bronchial Asthma

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### Abstract

#### Background

Endothelial Cell-Specific Molecule-1 (ESM-1) is a 50 kDa soluble proteoglycan that is produced mainly the vascular endothelial cells of the kidney and lung. It is produced by the effects of proangiogenic and pro-inflammatory cytokines, and it indicates activation and dysfunction of the vascular endothelium. We aimed to detect the role of ESM-1 in children with asthma.

#### Materials and Methods

This study is a prospective cross sectional study and include 50 child (32 with mild persistent, 18 with moderate persistent asthma (patients) and 30 healthy children served as controls, both groups were selected from outpatient pulmonology clinic and inpatient pediatric department at children hospital, Minia University, Egypt, from 2016 to 2018; and were subjected to: detailed clinical examination, lung function test, complete blood picture and measurement of level of ESM-1 in serum.

#### Results

Level of ESM-1 was increased in asthmatic children compared to the controls ( $p=0.001$ ). Also, the level of ESM-1 in children suffering from moderate persistent asthma was markedly higher than those with mild persistent asthma ( $p=0.001$ ). In addition, ESM-1 level was positively correlated with eosinophil counts ( $r=0.79$ ,  $p=0.01$ ), but had negative correlation with lung functions FEV1 and PEFR ( $r=-0.89$ ,  $-0.84$ ,  $p=0.001$ ).

#### Conclusion

ESM-1 level was increased in asthmatic children suggesting that it may have a role in asthma, furthermore, it was associated with decreased lung function indicating that it is considered as an indicator of severe asthma.

**Key Words:** Asthma, Children, Egypt, ESM-1.

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## 1- INTRODUCTION

Asthma is the most chronic respiratory disease of children characterized by airflow obstruction, bronchial hyper-responsiveness, and frequent exacerbations (1). The underlying cause of asthma is unclear but many environmental and genetic factors are involved in the development of asthma (2). It is a worldwide disease affecting more than 300 million people (3). Frequent exacerbation of asthma is an important leading cause of morbidity and even mortality (4). Also, it has negative effects on the quality of life of children and their families (5). Endothelial cell-specific molecule-1 (ESM-1) is a newly discovered soluble 50 kDa proteoglycan that is found in human blood and produced mainly by lung and kidney vascular endothelial cells, it is also called endocan (6). Usually the level of ESM-1 in blood is low and may be undetectable in healthy individual (7).

Under the effect of inflammatory cytokines, like (IL-1, TNF- $\alpha$ ) many endothelial pathological changes occur that induce the secretion of ESM-1, the endothelial changes include vasodilation, edema, coagulopathy, ischemia, and even organ failure. Thereby levels of ESM-1 may be closely related to the severity of inflammation (8, 9). Also, it inhibits migration of white blood cells into the pulmonary vasculature causing more lung damage (10). Studies that investigate the role of ESM-1 in asthma are few so that this study was aimed to measure the level of ESM-1 in asthmatic children and correlate its level to the severity of asthma.

## 2- MATERIALS AND METHODS

### 2-1. Study Design

This study is a cross sectional one done at the Department of Pediatrics, Minia University, Egypt, from January 2016 till April 2018. The study included fifty asthmatic children diagnosed according to

Global Strategy for Asthma Management and Prevention Classification (2). Informed consent was obtained from the parents before the study. The protocol was done according to the local ethics committee of the faculty. Another 30 healthy children cross matched by age and sex served as control group. Children under 5 years of age or with severe asthma or with associated infection, congenital heart disease were excluded from the study. The asthmatic patients were divided into two groups according to type of asthma;

- Group (1): included 32 children with mild persistent asthma (14 males and 18 females), their ages ranged from 5-11.5 years.
- Group (2): included 18 children with moderate persistent asthma (8 males and 10 females) their ages ranged from 6.5-13 years (2).

### 2-2. Method

Both patients (n=50), and controls (n=30) were subjected to:

- Thorough history taking about the course of the disease and complete clinical examination particularly chest examination and assessment of anthropometric measurements.
- Pulmonary function test using spirometry; it was performed three times and the highest flow rate of PEF and FEV1 were recorded and were compared to predicted normal values.

### 2-3. Laboratory investigations

Under complete aseptic condition 3ml of venous blood was withdrawn and 0.5 ml was added to tube containing Ethylenediamine tetraacetic acid (EDTA) for complete blood count (Sysmex KX-21N, Japan), and the other 2.5 ml was allowed to clot and was then centrifuged at 2,500 g for 15 min and the resulting serum was stored at  $-70^{\circ}\text{C}$  for ESM-1 assay

using ELISA (ELISA; LUNGINNOV Systems, Lille, France) to measure ESM-1 according to the instructions of the manufacture (normal values were:  $0.93 \pm 0.3$  ng/ml).

### 2-4. Statistical Analysis

The entered data were analyzed using IBM SPSS statistics (version 17; SPSS for Windows; SPSS Inc., Chicago, Illinois, USA). The Chi square test was used to compare between two variables or more. P-values less than 0.05 were statistically significant and Pearson's correlation coefficient (r) test was used for correlating data.

### 3- RESULTS

**Table.1** summarizes the demographic and laboratory data of the studied groups and it is shown that positive family history of asthma and atopy was 40% and 26%,

respectively. Eosinophil counts were increased significantly in children with asthma compared to the control ( $p < 0.01$ ). The lung functions (PEFR and FEV1) were markedly decreased in patients compared to control group ( $p < 0.001$ ). In addition, the level of ESM-1 was increased significantly in asthmatic patients compared to the control group ( $p < 0.001$ ).

The study revealed that ESM-1 level among patients sub-group was significantly increased in group I (patients with mild persistent asthma), and group II (patients with moderate persistent asthma) when compared to controls (group III) ( $p = 0.01$  and  $p = 0.001$ , respectively) (**Table.2**). Furthermore, the level of ESM-1 was markedly increased in group II (moderate persistent) compared to group I (mild persistent) ( $p = 0.001$ ) indicating that its level increases with increasing asthma severity.

**Table-1:** Demographic and laboratory findings of patients and control groups.

Variables	Asthmatic children n= 50	Control n=30	P- value
Age (y): Range	5.5-13	5-12	0.21
Mean $\pm$ SD	$4.2 \pm 2.5$	$4.5 \pm 1.6$	
Gender: Male	32	16	0.23
Female	18	14	
Weight (kg)	$25.2 \pm 8$	$25.6 \pm 7.2$	0,22
Weight/age percentile	$54.3 \pm 28.4$	$55.3 \pm 28.1$	
Height (cm)	$128.4 \pm 11$	$131.2 \pm 10$	0.36
height/age percentile	$42.4 \pm 24.5$	$43.2 \pm 24.6$	
BMI value	$18.2 \pm 3.5$	$18.3 \pm 1.5$	0.42
Positive family history of asthma	20 (40%)	0	
Positive family history of atopy	13 (26%)	0	
PBE count (cell/ $\mu$ l)	$570 \pm 120$	$128 \pm 66$	0.01
Percentage of PEFR I	$76.2 \pm 3.2$	$96.7 \pm 0.9$	0.01
Percentage of FEV1	$78.2 \pm 4.6$	$97.1 \pm 0.58$	0.01
ESM-1 level (ng/ml)	$25.2 \pm 4.3$	$4.2 \pm 2.5$	0.001

BMI=body mass index; PEFR=peaked flow rate; FEV1 =forced expiratory volume in 1st second; ESM-1: Endothelial cell-specific molecule-1; SD: Standard deviation.

**Table-2:** Comparison between patient's subgroup and controls.

Data	Group I n=32	Group II n=18	Control group (III), n=30	P- value		
				I vs. III	II vs. III	I vs. II
Eosinophil count (cell/ $\mu$ l)	433 $\pm$ 102	525 $\pm$ 29	128 $\pm$ 66	0.05	0.001	0.05
% PEFR predicted normal	85.9 $\pm$ 3.8	72.8 $\pm$ 9.7	96.7 $\pm$ 0.9	0.01	0.001	0,01
% FEV1 predicted normal	82.5 $\pm$ 1.7	70.8 $\pm$ 6.7	97.1 $\pm$ 0.58	0.01	0.001	0.001
Serum ESM-1 level (ng/ml)	3.2 $\pm$ 1.3	6.5 $\pm$ 0.9	0.93 $\pm$ 0.3	0.01	0.001	0.001

Group I: mild persistent; Group II: moderate persistent; Group III: controls; PEFR: peaked flow rate; FEV1: forced expiratory volume in 1<sup>st</sup> second; ESM-1: Endothelial cell-specific molecule-1.

**Table.3** demonstrates the correlation between ESM-1 and laboratory findings, level of ESM-1 was positively correlated with eosinophil count ( $r=0.79$  and  $p=0.01$ ),

and correlated negatively with the functions of the lung (FEV1 and PEFR ( $r=- 0.89$ , and  $-0.84$ , respectively  $p=0.001$ ).

**Table-3:** Correlation between ESM-1 level, Eosinophil counts and lung functions in asthmatic patients.

Parameter	Serum ESM-1 level	
	Pearson's correlation	P-value
Eosinophil count (cell/ $\mu$ l)	0.79	0.01
% of FEV1 predicted normal	-0.89	0.001
% PEF predicted normal	-0.84	0.001

ESM-1: Endothelial cell-specific molecule-1; PEFR: peaked flow rate; FEV1: forced expiratory volume in 1<sup>st</sup> second.

#### 4- DISCUSSION

Bronchial Asthma in children is considered as a major public health problem worldwide with broad differences in prevalence and severity throughout the world (11, 12). Endothelial cell-specific molecule-1 is a soluble 50 kDa dermatan sulfate proteoglycan that is produced mainly by vascular endothelial cells of the lung and kidney (13, 14). It has been found that ESM-1 can bind directly to lymphocyte function-associated antigen-1 (LFA-1) in vitro and can block binding of bacteria to the intercellular adhesion molecule-1 (ICAM-1) (15, 16); this may reduce leukocyte-endothelial cell adhesion, and inhibit the excessive leukocyte homing into the lungs. Some authors suggested

that ESM-1 may be considered as a good indicator of endothelial dysfunction and multiple-organ dysfunction in inflammation. Studies that investigate the role of ESM-1 in childhood asthma are scarce so this study aimed to determine whether ESM-1 has a significant role in asthma and, in addition, correlate it with asthma severity. In our study there were no significant differences in the anthropometric measurements between asthmatic children and control ( $p>0.05$ ); as regards eosinophil count, it was significantly increased in asthmatic children compared to controls ( $p=0.01$ ), and also it was significantly higher in children with moderate persistent asthma compared to those with mild persistent

asthma ( $p=0.001$ ). In addition, the lung functions (PEFR and FEV1) were markedly decreased in asthmatic children compared to controls ( $p= 0.001$ ). In the present study, serum level of ESM-1 was significantly increased in children with asthma compared to the control group ( $p=0.001$ ). Also, level of serum ESM-1 level in children with moderate persistent (group II) was significantly higher than those with mild persistent asthma (group I) control group ( $p <0.001$ ). The increased plasma level of may result from the effect of inflammatory mediators that were released during the inflammatory process of asthma like tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1. Also, Long, E.O. (17) found in vitro that, bacteria endotoxin, IL-1 and TNF- induce the synthesis and the release of ESM-I by HUVECs. These results were in agreement with Tang et al. (18), Abdelhalim and Elsayed (19) who found elevated plasma level of ESM-1 in patients with respiratory distress. Also, the higher level of ESM-I in children with moderate persistent asthma compared to those with mild persistent asthma may due to increased and sustained inflammatory process in patients with more severe asthma. This is in agreement with Tang et al. (18), who found that cases with severe respiratory distress have a sustained release of ESM-I and are associated with poor outcomes; therefore, ESM-I represents a good marker of endothelial cell dysfunction. In contrast, Mikkelsen et al. (20), reported lower serum levels of ESM-I in patients with acute lung injuries; they explained that it was associated with ESM-I -mediated blockade of leukocyte homing in the lung, although trauma and infection may differ clinically and biologically (21). In the present study, there was a positive correlation between serum levels of ESM-1 and the severity of asthma, and PBE count as an inflammatory marker of asthma may suggest that ESM-I is more than a simple marker of asthma and it may

play a role in systemic inflammatory process in asthmatic children. This may be explained by the findings of altered small airway wall vascularity and functional changes of the endothelial wall that occur with bronchial obstruction (22, 23). In addition, in asthma with recurrent exacerbations, the inflammatory changes observed during the course of each episode, including the levels of markers such as TNF- $\alpha$  and IL-6, have been shown to be of potential value in the exacerbation (24, 25). Also, vascular change that takes place during the inflammatory process of asthma such as microvascular hyperpermeability, vascular remodeling that involves the whole bronchial tree, increase in subepithelial blood flow and endothelial dysfunction (26, 27). There are adequate grounds for future studies of its role in the natural history of obstructive pulmonary diseases as well as in the follow-up of affected patients (28, 29). The relationship of the ESM-1 levels and the clinical outcomes of patients could be of great importance, possibly in line with recent findings supporting a close relation between serum levels of ESM-1 and disease severity (30, 31). We have demonstrated that ESM-1 can predict the severity of asthmatic attack. It may be a guide for further effective therapies. In addition, combined clinical variables with biological biomarkers such as ESM-1 may play an important role in early therapeutics or preventative approaches for asthma.

#### **4-1. Study Limitations**

First, the small number of patients included in the study, especially those with moderate persistent asthma. It may be useful to repeat the study on a larger sample of patients in future. Second, ESM-1 was measured only initially, at the time of admission, and the dynamics of concentration during the asthmatic evolution has not been evaluated so follow up sample must be taken into consideration.



## 5- CONCLUSION

Serum level of ESM-1 markedly increased in children with asthma, therefore it may play a role in systemic inflammatory process in asthma. Also, higher levels were associated with poor lung functions denoting that it may be considered as a marker of severity of asthma further studies are needed to confirm the role of ESM-1 in asthmatic children and to detect its role in other allergic diseases.

**6- CONFLICT OF INTEREST:** None.

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