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Endothelial dysfunction in children with clinically stable and exacerbated asthma

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Abstract

Introduction: In children with asthma, endothelial dysfunction signs are observed, and their extent depends on the severity of the disease. These changes are also present in remission. High level of soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) expression causes active adhesion of inflammatory cells and can indicate direct endothelium participation in development and supporting of chronic inflammation. Bronchial asthma (BA) is characterised by airways chronic inflammation. A special role in this inflammatory process formation is played by development of endothelial dysfunction.

The aim of the study was to evaluate endothelial state in children with clinically stable and exacerbated asthma.

Material and methods: 91 children with persistent asthma were examined. Among them there were 40 patients with mild persistent (group I), 34 subjects with moderate persistent (group II) and 17 individuals with severe persistent (group III) asthma. 20 healthy children were selected as controls. The serum levels of sVCAM-1 were determined by enzyme-linked immunosorbent assay (ELISA). The ultrasound assessment of endothelium-dependent flow-mediated dilation of the brachial artery (FMD%) has been made. Ultrasonography has been used for investigation of the intima-media thickness (I-M) complex. Data analysis was performed with the Statsoft Statistica Version 8 (Tulsa, OK).

Results: The serum levels of sVCAM-1 were significantly increased in the patients with asthma exacerbation ($p < 0.001$) and remission ($p < 0.001$), compared with the controls. The index of FMD% was significantly diminished in the patients of I, II, III group with exacerbation ($p < 0.001$) and stayed lower in the subjects with asthma in remission ($p < 0.001$), compared with the controls. The thickness of I-M complex was significantly increased in the patients of I, II, III group, compared with the controls ($p < 0.001$). The endothelium parameter levels: sVCAM-1 ($H = 56.11$, $p = 0.0001$), FMD% ($H = 43.20$, $p = 0.0000$), the thickness of I-M complex ($H = 49.37$, $p = 0.0000$) depend on the severity of the disease. Correlations between the endothelium and pulmonary function parameters were proved ($p < 0.05$).

Conclusions: Endothelial dysfunction in children with asthma was determined. Dependence of severity of the disease on functional state of the vascular endothelium was proved.

Key words: bronchial asthma, endothelium, inflammation, children

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Introduction

Being considered one of the most widespread chronic conditions among children, nowadays, asthma remains a global problem of health care [1–4]. In the world, there is a tendency to increase the number of patients with heavy and complicated bronchial asthma (BA) course [1]. It leads to considerable restrictions in physical, emotional and social aspects of human life [5, 6].

It is known that asthma is a chronic respiratory disease characterised by variable airflow obstruction and airway hyperresponsiveness [7–9]. Symptoms include wheezing, shortness

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of breath, chest tightness, and cough, ranging in severity from mild symptoms to life-threatening exacerbations [10]. Last years many researchers considered that this process is not limited to the lungs, but has systemic character [11]. Asthma is a heterogeneous group of conditions [12]. It has also been associated with procoagulant and antifibrinolytic activity in the airways [13, 14]. It is supposed that the risk of developing pulmonary embolism is significantly increased in asthmatic patients, compared to the general population. Frequent asthma exacerbations and hospitalisations are significantly associated with pulmonary embolism risk. It can be explained by the interaction between the inflammatory and haemostatic systems in asthma and other inflammatory diseases [15, 16].

When studying asthma formation and progress, it is necessary to consider an endothelial component state. The endothelium is a selectively permeable barrier between the vascular wall and bloodstream. In noninflamed tissue, it is responsible for maintaining blood fluidity and regulating blood flow and is able to control the vascular wall permeability, circulating leukocytes. The endothelium is also one of the first protective barriers against foreign invasion [17]. It performs a set of powerful functions, including barrier, transport, synthesis of proteins and vasoactive substances, takes active part in angiogenesis, coagulation processes, regulates a vascular tone [18–21]. The endothelium participation in asthma inflammatory process formation in children is of special interest in recent years. The mechanisms of inflammation cells migration from the blood stream to the bronchopulmonary inflammation site and also a role of endothelial cells and their molecular structures in this process are actively studied. For example, increased plasma cellular fibronectin in asthma has been shown, which is associated with the disease severity, inflammation, and prothrombotic blood alterations [22].

A special role in asthma inflammatory process formation is played by intercellular interrelations [23]. Basophiles, eosinophils and neutrophils are considered fundamental effector cells, which get into the asthmatic airways [23, 24]. Analysing inflammation from the point of view of leukocytes tissue-infiltration, we can note that mediators which influence endothelial cells also affect leukocytes, and vice versa. So microvascular endothelial cells in the inflammation site are active participants and inflammatory processes regulators [25]. In inflammation, endothelial cells activation induces increased vascular permeability for plasma proteins, the expression of proinflammatory cytokines, chemokines and enzymes, and the upregulation of adhesion molecules. Nuclear transcription factor-κB regulates the expression of adhesion molecules, such as intercellular adhesion molecule1 (ICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), and E-selectin that play a pivotal role in leukocyte-endothelium interactions [17]. sVCAM-1 is a well-known biomarker of endothelial activation and dysfunction [26].

There are many studies of endothelial dysfunction and sVCAM-1 involvement in its development. However, its role in the formation and maintenance of chronic inflammation in children with asthma is not fully understood.

In this research we evaluated endothelial state in children with clinically stable and exacerbated asthma.

Material and methods

General information

Ninety one children with persistent asthma exacerbation were examined. The tests were carried out on the first day of hospitalisation before the beginning of treatment. Clinically stable children were checked over in the same manner. All patients were divided into three groups according to the severity of asthma. Among them there were 40 children with mild persistent asthma (I group), 34 patients with moderate persistent asthma (II group) and 17 subjects with severe persistent asthma (III group). Twenty healthy children were randomly selected as controls, including 11 males and 9 females, with an average 10.03 ± 2.35. There were no significant differences in sex and age in all groups ($p > 0.05$). The patients with congenital respiratory diseases, hereditary diseases of the pulmonary system, cystic fibrosis, pneumonia and other acute or chronic inflammatory diseases were excluded. Also, the children who received antileukotriene medications and oral corticosteroids were eliminated. The diagnosis, criteria of severity of asthma, basic therapy were determined according to the recommendations of GINA 2017, National Asthma Education and Prevention Program's Expert Panel Report. Asthma control was quantified with the Asthma Control Questionnaire (ACQ) [27]. For the first time the children were examined when asthma exacerbation was confirmed. These patients returned in 4 weeks and the re-examination was conducted. The children were evaluated

Table 1. General information of patients with asthma

	I group mild persistent asthma n = 40	II group moderate persistent asthma n = 34	III group severe persistent asthma n = 17	Controls n = 20
Age, years mean ± SD	10.78 ± 3.62	10.75 ± 2.83	10.94 ± 3.14	10.83 ± 2.96
Male/Female	22/18	18/16	7/10	11/9
Atopy	22 (55%)	16 (47%)	14 (82%)*	–
Asthma duration (years) median (IQR)	3 (1; 4)	5 (2; 7)	9 (6; 11)*	–
Number of subjects prescribed ICS without LABA (percentages)	40 (100%)	24 (71%)	15 (88%)	–
Number of subjects prescribed ICS with LABA (percentages)	–	10 (29%)	2 (12%)	–
ACQ score	0.12 (0.10–0.35)	0.54 (0.48–1.15)	1.11 (0.57–1.79)*	–
Pulmonary function median (IQR) in exacerbation				
FEV_1, %	74.5 (69.0; 79.0)	67.5 (60.0; 74.0)	70.0 (62.0; 79.0)	–
FEF25, %	61.5 (51.5; 70.5)	38.5 (35.0; 43.0)	39.0 (29.0; 78.0)	
FEF50, %	68.0 (59.0; 72.5)	59.5 (52.0; 64.0)	58.0 (43.0; 73.0)	
FEF75, %	62.5 (53.5; 70.5)	57.0 (52.0; 62.0)	59.0 (42.0; 72.0)	
clinically stable FEV_1, %	94.0 (85.0; 100.5)	85.5 (80.0; 91.0)	81.0 (79.0; 86.0)	
FEF25, %	87.0 (80.0; 94.5)	92.0 (86.0; 97.0)	79.0 (70.0; 85.0)	
FEF50, %	87.5 (81.0; 95.5)	91.0 (85.0; 96.0)	82.0 (77.0; 87.0)	
FEF75, %	88.5 (82.0; 95.0)	86.0 (81.0; 92.0)	84.0 (79.0; 89.0)	

*$p < 0.05$ FEV_1: forced expiratory volume in 1 second; ICS: inhaled corticosteroids; LABA: long acting beta agonist; ACQ: scores on the Asthma Control Questionnaire range from 0 to 6, with lower scores indicating better asthma control and 0.5 as the minimal important difference; IQR: interquartile ranges; FEF: forced expiratory flow

during a research-only outpatient visit that was rescheduled if the following were reported within the preceding 2 weeks: 1) upper respiratory viral symptoms such as rhinorrhea, 2) an acute worsening of asthma symptoms, 3) antibiotic use, or 4) systemic glucocorticoid use (Table 1).

Ethics approval and consent to participate

The study was approved by the ethics committee of the Kharkiv National Medical University, Ukraine. Information consents were signed by all participants of the study before it began. All participants and/or their parents gave written informed consent.

Functional state of the endothelium

Venous blood (2 mL) was taken from the examined subjects in the morning following fasting and centrifuged at 2,000 × g for 10 min. The serum at the lower part of the test tube was stored at -20 °C. The collected blood samples were assayed for the serum levels of soluble Vascular Cell Adhesion Molekule-1 (sVCAM-1). sVCAM-1 was determined with enzyme-linked immunosorbent assay (Human sVCAM-1 Platinum ELISA, eBioscience, Bender MedSystems GmbH, Austria, BMS232. The samples for ELISA were duplicated). Endothelial function was evaluated with the help of digital ultrasonic diagnostic complex "Ultima PA" using a 10–15 MHz sensor. The ultrasound assessment concerned endothelium-dependent flow-mediated dilation of the brachial artery [28] and calculation of percentage increase in the brachial artery diameter (FMD%). FMD% is considered normal for > 10% [28]. Ultrasonography has been used for investigation of the intima-media thickness (I-M) complex of the common carotid artery.

The pulmonary function test (PFT) Spirometry was performed using the computerised spirometer SpiroCom "KhAImedica".

Statistical analyses

Statistical analyses were carried out with StatSoft STATISTICA Version 8 (Tulsa, OK). Para-

metric variables are presented as mean ± SD. Non-parametric variables are given as median (interquartile range). The Kruskal-Wallis One Way Analysis of Variance (ANOVA) on Ranks (H) was used for testing a statistically significant difference in the median values among all groups. Significance point was defined using Bonferonni adjustment. To compare two independent samples, non-parametric Mann-Whitney U-test was applied. To contrast the two dependent samples, the non-parametric Wilcoxon test (T) was used. The correlation between endothelial function parameters was determined using the Spearman rank correlation analysis (r); $p < 0.05$ was considered to indicate a statistically significant difference.

Results

sVCAM-1 levels

The serum levels of sVCAM-1 were significantly increased in the patients of I, II, III group with asthma exacerbation ($p_{controls\text{-}I} = 0.0000$; $p_{controls\text{-}II} = 0.0000$; $p_{controls\text{-}III} = 0.0000$) and remission ($p_{controls\text{-}I} = 0.0000$; $p_{controls\text{-}II} = 0.0000$; $p_{controls\text{-}III} = 0.0000$), compared with the controls. It was proved that the levels of sVCAM-1 depend on asthma exacerbation severity ($H = 56.11$, $p = 0.0001$; $p_{I–II} = 0.0000$; $p_{I–III} = 0.0000$; $p_{II–III} = 0.0000$) and remission periods ($H = 50.68$, $p = 0.0000$; $p_{I–II} = 0.0000$; $p_{I–III} = 0.0000$; $p_{II–III} = 0.0000$). The levels of sVCAM-1 significantly decreased in remission, compared with exacerbation ($p = 0.0000$, $T = 0$).

FMD%

The index of FMD% was significantly decreased in the patients of I, II, III group with exacerbation ($H = 43.20$, $p = 0.0000$; $p_{I–II} = 0.5633$; $p_{I–III} = 0.0135$; $p_{II–III} = 0.0038$; $p_{controls–I} = 0.0000$; $p_{controls–II} = 0.0000$; $p_{controls–III} = 0.0000$) and remission ($H = 46.02$, $p = 0.0000$; $p_{I–II} = 0.2952$; $p_{I–III} = 0.0010$; $p_{II–III} = 0.0005$; $p_{controls–I} = 0.0000$; $p_{controls–II} = 0.0000$; $p_{controls–III} = 0.0000$), compared with the controls. When contrasting FMD% in the patients with asthma in periods of exacerbation and remission, the lowest levels were found in the subjects in the time of exacerbation ($p < 0.001$, $T = 0$).

The thickness of I-M complex

The thickness of I-M complex was significantly increased in the patients of the I, II and III group with asthma exacerbation ($H = 49.37$, $p = 0.0000$; $p_{I–II} = 0.0046$; $p_{I–III} = 0.0000$; $p_{II–III} = 0.0055$; $p_{controls–I} = 0.0000$; $p_{controls–II} = 0.0000$; $p_{controls–III} = 0.0000$), compared with the controls. The thickness of I-M complex significantly diminished in all children with asthma in remission ($p = 0.0000$, $T = 0$), however, compared with the controls, it stayed higher ($H = 60.75$, $p = 0.0000$; $p_{I–II} = 0.0019$; $p_{I–III} = 0.0000$; $p_{II–III} = 0.0000$; $p_{controls–I} = 0.0000$; $p_{controls–II} = 0.0000$; $p_{controls–III} = 0.0000$).

Correlation between endothelial function parameters

The correlations between the levels of sVCAM-1 and FMD%, I-M complex during exacerbation ($r = –0.81$; $r = +0.80$, respectively ($p < 0.001$)) and remission ($r = –0.45$; $r = +0.75$, respectively [$p < 0.001$]), were found (Table 2).

Significant dependency of parameters of PFT (FEV_1, FEF_{25}, FEF_{50}, FEF_{75}) on sVCAM-1 level and other functions of the endothelium is proven by existing correlations in the period of exacerbations and in remission phase (Table 3). Thus, the higher are the levels of sVCAM-1 in blood serum, the worse are the values of external respiration function, especially those ones which are specific for asthma.

Discussion

Deterioration in elastic properties of the vessel wall and endothelium structural violations in children with asthma, both in the exacerbation period and beyond its active process, is firmly established. It can be an adverse sign of a pathological process course. Dependence of bronchoobstructive syndrome manifestation extent from a vessels' endothelium functional state is confirmed by the existence of reliable correlation with pulmonary function parameters.

In our study, it has been shown that with an increase of disease severity, also the degree of endothelial dysfunction grows. The most expressed changes are established in children with severe asthma.

The endothelium is one of the first protective barriers against foreign invasion and when the endothelium senses stimuli, it releases factors to regulate haemostasis, cell growth, vasomotor function, and inflammatory processes [17]. Developing endothelium dysfunction is followed by endothelial cells activation, which in turn express mediators of inflammation and adhesion molecules [29, 30].

The key role in cellular homeostasis regulation and also in implementation of many effector cellular functions is played by intercellular interactions whose regulation is mainly carried out by

Table 2. Endothelial function parameters in the four groups(Me (Lq; Uq))

Endothelial function parameters	I group mild persistent asthma n = 40	II group moderate persistent asthma n = 34	III group severe persistent asthma n = 17	Controls n = 20
		Exacerbation		
FMD%	7.14 (6.25; 9.52)	7.89 (5.88; 8.88)	5.55 (3.22; 6.66)	23.33 (17.07; 27.77)*
The thickness of I-M complex, mm	0.9 (0.8; 1.0)	1.0 (0.9; 1.2)	1.2 (1.1; 1.3)	0.6 (0.5; 0.7)*
sVCAM-1, ng/ml	990.27 (900.52; 1080.89)	1280.00 (1100.27; 1380.73)	1700.73 (1480.27; 1920.59)	730.01* (690.63; 790.19)
		Clinically stable		
FMD%	11.27 (8.28; 13.33)	10.12 (8.88,11.76)	8.33 (6.45; 8.82)	23.33* (17.07; 27.77)
The thickness of I-M complex, mm	0.8 (0.8; 0.9)	1.0 (0.8; 1.1)	1.1 (1.1; 1.2)	0.6 (0.5; 0.7)*
sVCAM-1, ng/ml	885.42 (800.57; 990.47)	1150.43 (990.37; 1280.77)	1500.18 (1300.32; 1700.25)	730.01* (690.63; 790.19)

*$p < 0.001$

Table 3. Spearman's linear correlation analysis of endothelial functions and external respiration function in the children with asthma in exacerbation and clinically stable

Exacerbation			
Pulmonary function	**% FMD**	**The thickness of I-M complex**	**sVCAM-1**
FEV_1	+0.45*	–0.22	–0.51*
FEF_{25}	+0.26	–0.19	–0.14
FEF_{50}	+0.39	–0.43	–0.34*
FEF_{75}	+0.41*	–0.39*	–0.48*
Clinically stable			
FEV_1	+0.28	–0.36	–0.45*
FEF_{25}	+0.31*	–0.25	–0.29
FEF_{50}	+0.35*	–0.48*	–0.25
FEF_{75}	+0.38*	–0.41*	–0.50*

*$p < 0.05$

means of cell adhesion molecules and mediators of inflammation [31, 32].

Inflammation leads to endothelial cells activation which are active participants and regulators of inflammatory process, and express intercellular adhesion molecules, including sVCAM-1 [17]. Further, it promotes an increase in vascular permeability and active migration of leukocytes in the vessel wall [17]. It is assumed that the «intima-media» complex is thickened because of adhesion to vascular endothelium of leukocytes, with participation of sVCAM-1. Therefore, the IMC thickening occurs, which we have also established.

Thus, sVCAM-1 expression by various cells forms the pathological process leading to permanent changes in the vessels' endothelium and is one of chronic inflammation formation mechanisms, as it has been shown in this research.

Besides, endothelial cells effectively induce CD4 and CD8 memory T-cells to proliferation and production of cytokines. It promotes accumulation of oxygen active forms, and consequently further pathological reactions leading to inflammation. All these processes involve a vasodilatation decrease, development of pro-inflammatory states and proliferative changes in the wall of vessels [17]. There is the endothelium hypertrophy and hyperplasia, both due to inflammatory changes, and to oxidative stress that leads to thickening of the intima and media, and consequently to the continuing violations of adequate endothelium functional activity [19].

In conclusion, endothelial dysfunction in children with asthma was determined in exacerbation and remission. A degree of endothelial dysfunction depends on the severity of the disease. Expression of sVCAM-1 pathological process development which leads to changes in the vascular endothelium can be one of the mechanisms of inflammation formation. The established interrelationship of endothelial function parameters, and specifically sVCAM-1, the thickness of I-M complex, FMD % with pulmonary function parameters in the children with asthma in the period of exacerbation and remission, indicate

direct endothelium participation in development and supporting of chronic inflammation.

Authors' contributions

All authors were involved in planning and design of the study, data analysis, drafting and critical revision of the paper. Contribution of the authors was the following: Development of the clinical study design: Nataliia Makieieva, Dmytro Butov; Patient recruitment and clinical data analysis: Maryna Biriukova, Kateryna Serhiienko; Statistical analysis: Yuliia Vasylchenko, Oleksandr Morozov; Writing the paper: Yuliia Vasylchenko, Nataliia Makieieva. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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