

# Is Willebrand Factor Indicative of Chronic Inflammation in Children with Asthma?

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## Abstract

**OBJECTIVE:** To improve our knowledge and to understand how the level of von Willebrand factor indicates the development of chronic inflammation in children with recurrent wheezing and asthma.

**MATERIAL AND METHODS:** It was a prospective cohort study. This study was conducted in children with recurrent wheezing and asthma who were referred to a children's hospital during 2017-2018. Patients were divided into 3 groups depending on the number of episodes of wheezing. Patients were examined for von Willebrand factor levels at admission and after treatment. Data analysis was performed with Statsofta Statistica Version 8 (Tulsa, OK).

**RESULTS:** WF1 levels in Group 2 and 3 children statistically significantly increased in comparison with the control group ( $p < 0.001$ ). WF2 levels remained elevated only in Group 3 patients ( $p < 0.001$ ). WF2 levels in Group 1 and 2 decreased to the indices of the control group ( $p > 0.05$ ). The WF2 significantly decreased after treatment in Group 2 children ( $p = 0.0000$ ,  $T = 0$ ) and Group 3 ( $p = 0.0000$ ,  $T = 0$ ).

**CONCLUSION:** levels of Willebrand factor indicate the presence of endothelial dysfunction. The level of Willebrand factor in the peak period of wheezing depends on the number of episodes of wheezing in history. Persistent high rates of Willebrand factor, even after the relief of clinical symptoms, indicates the present of chronic inflammation and can be regarded as the formation of asthma in children.

**KEYWORDS:** Asthma, endothelium, children, willebrand factor, inflammation

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## INTRODUCTION

Presently, asthma is a global problem. It is a chronic disease of the bronchopulmonary system and affects almost 300 million people worldwide [1-4]. The disease usually first occurs in childhood and develops until the patient is 6 years old [5-7]. The onset of asthma, which is clinically manifested in the form of wheezing, is often regarded as a syndrome of other pathologies [6]. Presently, it remains important to determine the formation of the disease in children of this age cohort and to study in depth the methods for diagnosing chronic inflammation of the respiratory organs. Chronic inflammation, which is the main feature in the pathogenesis of asthma, affects all layers of tissues that form the system as a whole. Chronic inflammation affects the vascular endothelium, the blood coagulation system, the complement system, and others [4, 8]. Assessing the literature, endothelial dysfunction was detected in various diseases [cardiovascular, endocrine, etc], and pro-inflammatory and anti-inflammatory cytokines are a marker for their detection [4, 8, 9].

According to sources, the Willebrand factor can be considered as a potential marker of endothelial dysfunction as the synthesis of the von Willebrand factor occurs through the vascular endothelium, and various changes in its state manifest in its concentration in the plasma [10, 11]. Given these data, the study of endothelial dysfunction markers in asthma and repeated rates can reveal new information about the development of chronic inflammation and the occurrence of asthma. Presently, the features of functional state of the endothelium at different stages of asthma have conflicting data, especially among the cohort of young children with the onset of the disease. In this study, we conducted a comparative assessment of the state of the vascular endothelium by determining the levels of the Willebrand factor in the blood plasma of children with recurrent episodes of wheezing and with an exacerbation of asthma.

## MATERIAL AND METHODS

### Subject (Study Population)

It was a prospective cohort study. The study was conducted from March 2017 to October 2018 in the Children's Clinical Hospital. A sample of 302 children, aged 1-6 years, with asthma and recurrent wheezing was taken; they were all seen

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by a pulmonologist or were treated for repeated wheezing in the hospital. A total of 81 children were randomly selected and invited to participate in the study. The patients underwent specific treatment according to WHO recommendations [12]. All patients' clinical medical history was recorded, physical examination was conducted, and laboratory assessments were obtained. The control group comprised 15 healthy children [of similar age and gender] who had no signs of chronic diseases and no signs of acute diseases for the past three months and presented to the hospital for routine health control or vaccination. The parents of all patients and controls were informed about the study objectives, and written informed consent was obtained before inclusion in the study.

All patients and healthy children were examined. Patients were divided into groups depending on the number of episodes of wheezing per their history. Among the 81 patients, there were 18 patients with a history of two episodes of wheezing; 32 patients with a history of three to five episodes of wheezing; 31 patients with a history of more than five wheezing episodes more per their anamnesis. Patients in the group with more than five wheezing episodes had a diagnosis of asthma. The clinical diagnosis was verified per Global Initiative for Asthma (GINA) 2018 recommendations and included symptoms [cough and wheeze] for more than 10 days during an upper respiratory tract infection, more than three episodes of wheezing per year, and the child had a cough between episodes and had atopic dermatitis or food allergy or a family history of asthma.

Inclusion criteria were as follows: signing of informed consent by the patient's parents, age between 1 and 6 years, a history of preceding viral upper respiratory infection, established diagnosis of mild-to-moderate persistent asthma, using the Global Strategy for Asthma Management and Prevention guidelines developed by the Global Initiative for Asthma (GINA 2018), recurrent wheezing episodes according to the European Respiratory Society (ERS) classification. Exclusion criteria were as follows: congenital and chronic cardiopulmonary or neurological disease; hereditary diseases that lead to changes in the functioning of the respiratory tract, including cystic fibrosis; proven immune deficiency; proven or suspected acute bacterial infection; previous treatment with anti-leucotriene drugs or systemic corticosteroids; and patients receiving vasoactive agents less than 48 hours before the study.

#### MAIN POINTS

- Willebrand factor levels indicates the dysfunction.
- The level of Increase in levels of Willebrand factor in the blood plasma is determined in children with recurrent wheezing.
- Highest levels of Willebrand factor are determined in children with asthma.
- Persistent high rates of Willebrand factor indicates the presence of chronic inflammation and can be regarded as the formation of asthma in children.

The planned clinical studies were carried out after receiving approval by the local ethics committee (date: February 1, 2017; number: 2017/01) and were conducted in accordance with the principles of the Helsinki Declaration, amended in October 2013.

#### Determination of Willebrand Factor Levels

Willebrand factor levels were determined in the first two days of clinical manifestations of episodes of wheezing and exacerbation of asthma before the start of therapy (WF1) and when arresting clinical manifestations, after the treatment, on the 10<sup>th</sup>-14<sup>th</sup> day (WF2). The Willebrand factor was studied only at baseline in the control group.

The blood was collected in the morning, under fasting conditions, by venipuncture using a standard technique or from a venous catheter, if available. Blood was collected into special tubes with a K2ADTA or K3ADTA anticoagulant. The samples were then centrifuged for 10 min at 1500-2000 rpm. All plasma samples were frozen at -70°C until assay. Plasma Willebrand factor levels were measured by using a Ristocytin test (RISTO test), where after receiving platelet-free plasma by centrifuging with a 0.1% solution of ristocytin and its mixture at standard dilution, the index of ristocytin aggregation is determined, which reflects its concentration in blood plasma. Results were expressed as a percentage.

#### Statistical Analysis

All statistical analyses were performed using the StatSoft STATISTICA version 8 package program [Tulsa, OK]. A Shapiro-Wilk test was used, and a histogram and q-q plot were examined to assess the normality. Non-parametric data were expressed either as median [Me] interquartile range [Lq: lower quartile; Uq: upper quartile]. The sample size was estimated in the statistical program "StatSoft STATISTICA version 8" with a power of 90% and a type 1 error of 0.05%.

When comparing the indices, which were characterized by comparing more than two points, the Kruskal-Wallis dispersion analysis criterion (KW) was used, and the differences were considered reliable, taking into account the Bonferroni correction. For the comparison of indices of dependent samples, the Wilcoxon non-parametric criterion (t test) was used. Two independent samples were compared using the non-parametric Mann-Whitney test (MW). p values less than 0.05 were considered statistically significant. Correlations between the number of wheezing episodes [qualitative] and von Willebrand factor level [quantitative] were estimated using the biserial correlation coefficient [Rb].

#### RESULTS

All 81 patients who were included in the study were divided into groups depending on the nosologically form and the number of episodes of wheezing per their history. Group 1 included 18 patients who had no more than two episodes of wheezing, group 2 had 32 children with three or more episodes of wheezing, and group 3 involved 31 patients with asthma.

When comparing groups by age, sex, the need for oxygen therapy during the period of attack, and the indicators of

**Table 1.** The main group clinical and laboratory data

Sign	Group 1	Group 2	Group 3	p
Gender, M/F	10/8	18/14	18/13	>0.05
Age, years, Me [Lq; Uq]	3.27 [2.15; 4.1]	3.47 [2.87; 4.21]	3.57 [2.12; 5.11]	>0.05
Positive family allergic history	27% [5/18]	28% [9/32]	35% [11/31]	>0.05
Presence of atopic dermatitis in children	5.6% [1/18]	3.1% [1/32]	16.1% [5/31]	$p_{1,2}=0.3607$ $p_{1,3}=0.1550$ $p_{2,3}=0.0410$
Presence of food allergy in children	11.1% [2/18]	12.5% [4/32]	6.5% [2/31]	>0.05
Onset of wheezing of the first year of life	27.8% [5/18]	37.5% [12/32]	32.2% [10/31]	>0.05
Oxygen therapy	16.7% [3/18]	18.7% [6/32]	16.1% [5/31]	>0.05
Leukocytosis, %	11.1% [2/18]	3.1% [1/32]	9.7% [3/31]	>0.05
Lymphocytosis, %	83.3% [15/18]	87.5% [28/32]	80.1% [27/31]	>0.05
High eosinophil blood parameters, cells, $\mu$ l	11.1% [2/18]	15.6% [5/32]	38.7% [12/31]	$p_{1,2}=0.3145$ $p_{1,3}=0.0211$ $p_{2,3}=0.0224$
ESR increase, mm/h	33.3% [6/18]	37.5% [12/31]	28.1% [9/32]	>0.05
CRP, mg/l	22.2% [4/18]	18.8% [6/32]	22.6% [7/31]	>0.05
Glycoproteins increase, absorbance unit	27% [5/18]	25% [8/32]	22.6% [7/31]	>0.05
Seromucoid increase, absorbance unit	22.2% [4/18]	18.8% [6/32]	25.8% [8/31]	>0.05
Ig E increase, IU/mL	33.3% [6/18]	48.9% [15/32]	70.9% [22/31]	$p_{1,2}=0.2785$ $p_{1,3}=0.0127$ $p_{2,3}=0.0799$

**Table 2.** Statistical indices of the Willebrand factor

	WF at the beginning of wheezing, % Me [Lq; Uq]	WF after treatment of wheezing, % Me [Lq; Uq]
Group 1	97.80 [96.80; 98.50]	97.70 [96.00; 99.40]
Group 2	109.81 [105.80; 113.50]	97.92 [95.60; 100.95]
Group 3	133.24 [130.40; 139.50]	110.83 [101.20; 117.50]
Control	99.51 [95.40; 102.40]	99.51 [95.40; 102.40]
	KW: H=83.8862; p <0.001	KW: H=42.0083; p <0.001
	MW: $p_{1,2} <0.001$ ;	MW: $p_{1,2} = 0.0674$ ;
	$p_{1,3} <0.001$ ;	$p_{1,3} <0.001$ ;
	$p_{2,3} <0.001$ ;	$p_{2,3} <0.001$ ;
	$p_{1-control} =0.0963$ ;	$p_{1-control} =0.1116$ ;
	$p_{2-control} <0.001$ ;	$p_{2-control} =0.5453$ ;
	$p_{3-control} <0.001$ .	$p_{3-control} <0.001$ .

standard biochemical data, there are no statistically significant differences. In the anamnesis data on the presence of atopic dermatitis, laboratory data on elevated levels of total IgE and eosinophil levels were significantly more frequent in patients in group 3 (Table 1).

The Kruskal-Wallis test showed a statistically significant difference in WF1 levels between groups. It was revealed that WF1 levels in children of groups 2 and 3 had a statistically significant increase compared with the control group. The highest rates were observed in patients of group 3. Indicators of patients in group 1 did not differ from the control group. When

relieving wheezing, the Kruskal-Wallis test also showed significantly statistical differences between groups. WF2 levels remained elevated only in patients of the third group, while WF2 levels in the first and second groups decreased compared with those of the control group. Indicators of WF2, secondary to ongoing anti-inflammatory and bronchodilator therapy, decreased significantly over time in group 2 [ $p=0.0000$ ,  $T=0$ ] and in group 3 [ $p=0.0000$ ,  $T=0$ ] (Table 2). A reliable correlation between the number of wheezing episodes and the level of von Willebrand factor ( $R_b=0.83$ ,  $t=13.09$ ,  $p<0.001$ ) has been proved in biserial correlations.

## DISCUSSION

This study has several limitations. First, about 55% of children had a comorbid disease - atopic dermatitis or food allergies, which can influence the level of von Willebrand factor in blood plasma. Thus, it is possible that the level of von Willebrand factor that we obtained in children with asthma is too high due to concomitant allergic disease [13]. Second, we evaluated the severity and duration of the disease and the verification of the diagnosis of asthma only by clinical and anamnestic patient data [12].

The vascular endothelium is one of the protective barriers, and as inflammation develops, it reacts with functional disorders. When responding to pathological effects, the vascular endothelium releases many biologically active agents [14]. The modern view of the endothelium presents it as an active endocrine organ, which is distributed throughout all vessels and systems and is one of the first to respond to inflammatory processes in the body through its dysfunction [15, 16]. Studies have described and identified Willebrand factor levels as an indicator of endothelial dysfunction [10, 17, 18]. Studies of the non-hemostatic role of the Willebrand factor remain controversial and not fully understood. Currently, it is not known whether the increased expression of the Willebrand factor is a cause or consequence of endothelial dysfunction, but changes in its levels directly or indirectly indicate endothelial dysfunction [10]. Our study showed an increase in the levels of Willebrand factor at the beginning of the development of the wheezing, which indicated manifestations of endothelial dysfunction secondary to an active inflammatory process.

It is known that coagulation and anticoagulation are locally activated in the airways, especially in the inflamed airways, in such conditions as the acute respiratory distress syndrome, pneumonia, pulmonary fibrosis, and respiratory viral infections that have been studied and proven in the adult population [19]. In pediatric practice, studies have been conducted indicating that Willebrand factor levels are a marker of pulmonary endothelial damage in acute inflammatory lung damage, namely, pneumonia [20, 21] and acute respiratory distress syndrome [22].

Modern studies have shown changes in Willebrand factor indices in children as well as in asthma. This study was aimed at determining systemic blood coagulation during exacerbation and remission of asthma in children. Our study showed a significant increase in Willebrand factor levels in children with asthma, which is explained by the activation of the endothelium, resulting in the release of Willebrand factor [13, 23]. Moreover, our study identified more significant increases in Willebrand factor indices in children in the onset of clinical manifestations of asthma exacerbations compared with a less-pronounced increase in Willebrand factor levels in developed clinical manifestations during recurrent episodes of wheezing and the absence of changes in indices during the first episodes of bronchial obstruction, which may indicate the severity of the inflammatory process, depending on the number of episodes of recurrent wheezing in history.

Modern studies assessed changes in Willebrand factor indices in the evaluation of chronic inflammation in asthma. Despite its main function in hemostasis, its participation in asthma development has been shown, when activation occurs after exposure to a damaging factor during an asthma attack. An increase in Willebrand factor levels associated with the severity of the disease has been proved [22] and a decrease in indices has been shown in the period of remission [23]. In our study, children were examined at the stage of arresting clinical manifestations, namely, inflammation of the respiratory tract, Willebrand factor levels remained above the norm among children with asthma, suggesting that the pathological process was chronic. In addition, in patients who had a greater number of repeated wheezing episodes in history, higher levels of the Willebrand factor were noted, whereas in the first episodes, Willebrand factor levels decreased to the level of the control group. These results can be interpreted as the formation of more persistent changes in epithelial dysfunction among patients with recurrent wheezing episodes.

Thus, an increase in plasma Willebrand factor levels indicates the presence of endothelial dysfunction. The level of Willebrand factor in the blood plasma during the peak period of wheezing depends on the number of episodes of bronchial obstruction in history. Persistent high rates of Willebrand factor, even after the relief of clinical symptoms, indicates the presence of chronic inflammation and can be regarded as the formation of asthma in children.

**Ethics Committee Approval:** Ethics Committee approval for the study was obtained from the Local Ethics Committee of Kharkiv National Medical University (February 1, 2017; number: 2017/01).

**Informed Consent:** Written informed consent was obtained the patients before inclusion in the study.

**Peer-review:** Externally peer-reviewed.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

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