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ORIGINAL ARTICLE

Content of Markers of Respiratory Tract Remodeling in Exhaled Breath Condensate in Children Suffering from Bronchial Asthma

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Background: This study investigated to detect the content of remodeling markers of the respiratory tract in the exhaled breath condensate (EBC) of children with bronchial asthma (BA). **Materials and Methods:** The study was in clinical groups of children with BA were formed on the indices in sputum supernatant: the Group I - "a high risk group" of bronchial remodeling (37 patients with vascular endothelial growth factor (VEGF) more than 80 ng/ml, and matrix metalloproteinase-9 (MMP-9) more than 5.2 ng/ml), the Group II - "a moderate risk" of bronchial remodeling (41 patients with VEGF more than 80 ng/ml, and MMP-9 <5.2 ng/ml, or VEGF <80 ng/ml, and MMP-9 more than 5.2 ng/ml), the Group III - "a low risk" of bronchial remodeling (38 patients with VEGF did not achieve 80 ng/ml, and MMP-9 <5.2 ng/ml). **Results:** The results obtained enable to believe that in EBC of children with a high risk of bronchial remodeling was higher azoalbumin olysis (1.8 ml/h comparison of Group II - 1.5 ml/h, $P = 0.038$, and Group III - 1.4 ml/h, $P = 0.007$), high catalase activity (81.26 comparison of other group - 50.7, $P = 0.06$, and 47.07, $P = 0.052$ μmol/min/mg of protein). Furthermore, in the clinical Group I, the content of nitrogen monoxide metabolites (53.5 mcmol/L) comparison of Group II of children (48.7 mcmol/L, $P = 0.28$) and the Group III (41.7 mcmol/L, $P = 0.085$). **Conclusions:** In children with a "high risk" of respiratory tract remodeling more significant inflammatory process in the bronchi is found than in children with a "low risk."

Key words: Bronchial asthma, children, exhaled breath condensate, bronchial remodeling

INTRODUCTION

The term "remodeling" usually means changes in the structure of the tissue content occurring as a result of disorders of natural reparation mechanisms in case of their damage. As a rule, remodeling occurs in individuals susceptible to this process in case of chronic inflammation, tissue damage, or combination of these factors. The signs of stable vascular alteration of the respiratory tract in case of bronchial remodeling are determined by their hypertrophy and angiogenesis manifested by 2–3 times increased number of vessels in the area of small and middle-size bronchi.[1,2] Increased permeability of the vascular wall, stagnation phenomena, and increased general volume of the vascular bed are accompanied by bronchial obstruction, persisting inflammation, increased the temperature on the mucous surface with increased concentration of nitrogen monoxide in the exhaled breath. All these factors promote hyperactivity of the bronchi, their reduced function, intensified course of the disease, and in some extent, it influences upon the efficacy of treatment.[3-5] Thus, the aim of the research was to detect the content of remodeling markers of the respiratory tract in the exhaled breath condensate (EBC) of children suffering from bronchial asthma (BA).

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MATERIALS AND METHODS

To achieve the purpose, 116 children suffering from persisting BA were examined at the Allergological Department of the Municipal Clinical Hospital "Regional Children Clinical Hospital" in Chernivtsi (Ukraine) in parallel groups applying the method of simple random sampling. Three clinical groups were formed on the basis of detected concentrations of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) in sputum supernatant of the patients examined. The first (I) clinical group further marked as "a high risk group" concerning formation of bronchial remodeling included 37 patients with the content of VEGF in sputum supernatant more than 80 ng/ml, and MMP-9 more than 5.2 ng/ml (67.6% of boys with an average age 12.0 ± 0.46). The second (II) group marked as "a moderate risk group" of bronchial remodeling included 41 patients with VEGF level in sputum supernatant fluid more than 80 ng/ml, and MMP-9 <5.2 ng/ml, or VEGF content <80 ng/ml, and MMP-9 more than 5.2 ng/ml (61.0% of boys with an average age 11.5 ± 0.54). The third (III) clinical group included 38 patients with BA further marked as "a low risk group" of bronchial remodeling. The concentration of VEGF in sputum supernatant of this group did not achieve 80 ng/ml, and MMP-9 did not exceed 5.2 ng/ml (67.8% of boys with an average age of 11.2 ± 0.52). The duration of BA in the Group I of children was 5.8 ± 0.68 years, in Group II 4.4 ± 0.68 years, and in the low-risk group of bronchial remodeling – 4.4 ± 0.63 years (in all the cases $P > 0.05$). Therefore, the groups of comparison were compared by the main clinical characteristics.

To obtain sputum, the procedure of induction was carried out by means of inhalation of sodium chloride serial hypertonic solutions by Pavord *et al*.[6] Biomarkers of bronchial remodeling in sputum supernatant were detected in the following way: VEGF – by means of three-stage "sandwich"-variant of the solid-phase immune-enzyme analysis using monoclonal and polyclonal antibodies (reagents «VEGF-Vector-Best» A-8784, RF), MMP-9 – by means of "sandwich"-ELISA method (reagents "Affymetrix eBioscience" BMS 2016/2/BMS2016/2TEN (Bender MedSystems, GmbH, Austria).

EBC was collected by means of our designed and patented device in terms of recommendations.[7] During 10–15 min of free respiration, usually, 1.5–2.0 ml of EBC was obtained in which the following indices were detected: Total protein content (g/L) by Lowry's method, EBC proteolytic activity by the lysis of albumin, azocasein, and azocollagen by Veremeyenko's method, the content of aldehyde and ketone derivatives of 2,4-dinitrophenylhydrazone (AKDNPH) of the main and neutral character by Dubinina's method, the content of nitrogen monoxide metabolites (NMON) by Yemchenko's method (1994) in Gozhenko's modification (2002), total, enzymatic, and non-enzymatic fibrinolytic activity by Kukharchuk's method.

The results obtained were analyzed by means of the computer packages Statistica 6.0 and Excel XP for Windows using parametric and non-parametric calculation methods. Diagnostic value of the test was estimated considering sensitivity (Se), specificity (Sp), prognostic value of positive and negative results (PVPR and PVNR), likelihood ratio of the positive (LR+), and negative (LR−) results. In all cases, confidence 95% interval was detected (95% confidence interval [CI]). Risk assessment evaluation was carried out considering the values of relative, attributive risk, chance ratio with detection of their 95% CI.

RESULTS

According to the degree of severity BA in the clinical Group I of patients was distributed in the following way: severe form – in 13.5% of patients, moderate – in 62.2% of children and mild persisting – in 24.3% of patients. In the clinical Group II, severe variant of the disease constituted 19.5%, moderate – 41.5%, and mild – 39.0% (in all the cases $P > 0.05$). In the group of children with a low risk of bronchial remodeling, the patients with a severe persisting course of BA constituted 23.7%, moderate – 34.3%, and mild –42.0% (in all the cases $P > 0.05$). According to the form, BA was distributed in the following way: atopic form was found in 67.6% of patients in Group I, in 56.1% of children from the moderate risk group and in 63.2% of representatives of the Group III of comparison (in all the cases $P > 0.05$), a mixed form of the disease was found in the rest of children.

Table 1 presents the values of total protein content and products of its oxidative modification in EBC.

Therefore, an inconsiderable increase of the total protein concentration in EBC was found in the part of children that in

Table 1: The values of protein oxidative modification and the content of total protein in exhaled breath condensate of children

Clinical groups	Total protein (g/L)	AKDNPH of the main character (E370 mmol/g of protein)	AKDNPH of the neutral character (E370 mmol/g of protein)
Group I (*n*=37)	3.3±0.23	52.4±12.67	7.0±2.70
Group II (*n*=41)	3.8±0.29	65.7±9.66	6.3±0.71
Group III (*n*=38)	3.7±0.60	59.3±17.84	6.4±1.67
P	>0.05	>0.05	>0.05

AKDNPH=Aldehyde and ketone derivatives of 2,4-dinitrophenylhydrazone

general did not differ reliably from the value of the regional norm (3.3 ± 0.2 g/L). It can be explained by lower activity of an inflammatory process in the bronchi in children from the Groups I and II associated with reduced transudation of protein through the vascular wall. At the same time, an increased content of aldehyde and ketodinitrophenylhydrazones of a neutral character proves a higher activity of an inflammatory process in children from the Group I of comparison.

Table 2 presents proteolytic (ml/h) and fibrinolytic (μg azofibrin/ml per 1 h) EBC activity in children from the groups of observation.

The results obtained enable to believe that higher proteolytic and enzymatic EBC activity is found in children with a high risk of bronchial remodeling. EBC proteolytic activity according to azoalbumin olysis more than 1.8 ml/h possessed the following indices of diagnostic value concerning detection of a high risk of structural changes in the bronchi: Test sensitivity (TS) – 11.1% (95% CI 5.7–19.0), TS – 91.9% (95% CI 84.7–96.4) with predicted value of positive and negative results 57.2% (95% CI 33.0–79.3) and 51.3% (95% CI 43.8–58.8). Posttesting probability of a positive result became 7.7% increased, and in case of a negative result, it became 0.8% decreased. Therefore, in spite of high TS its diagnostic value, in general, was insignificant that was evidenced by probability indices of the positive (1.4) and negative (1.0) results.

At the same time, it should be noted that catalase activity performing the role of an extracellular "scavenger" achieved in an average 81.26 mcmol/min in the clinical Group I, in the children from the Group II 50.7, and in the Group III 47.07 mcmol/min/mg of protein ($P < 0.05$), which is indicative of less inflammation activity in patients from the clinical Groups II and III. Table 3 presents the diagnostic value of exhaled air condensate indices in BA patients with a high risk of bronchial remodeling.

Application of these tests to detect children with a high risk of bronchial remodeling increased LR in case of a positive result: For the content of total protein more than 4.0 g/L – on 5.8%, for the markers of protein peroxide oxidation of the main character of the above-mentioned value – on 3.9%, and for catalase activity indicated above – on 68.3%. In case of a negative result of the test probability of a high risk of bronchial remodeling development became 5.2%, 1.6%, and 4.8% decreased, respectively. Table 4 presents the indices of high-risk probability of bronchial remodeling in children.

The data presented are indicative of the fact that only catalase activity in EBC higher than the point of distribution is indicative of a probable risk of high likelihood concerning the development of structural changes in the bronchi.

Considering the fact that the content of nitrogen monoxide metabolites (NMOM) in EBC reflects the activity of an inflammatory process, especially in patients with eosinophilic bronchitis, making an analysis of this index seemed to be reasonable. Thus, its content in the I clinical group was 53.5 mcmol/L in an average, in the Group II of children – 48.7, and in the Group III – 41.7 mcmol/L ($P > 0.05$), which is indicative of a higher inflammation activity in patients from the clinical Groups 1 and II. It is an interesting fact that more

Table 2: The values of fibrinolysis and proteolysis in exhaled breath condensate of children from the groups of observation

Clinical groups	Values of EBC proteolytic activity			Values of EBC fibrinolytic activity		
	By albuminolysis	By azocasienolysis	By azocololyis	TFA	NFA	EFA
Group I (*n*=37)	1.8±0.02	1.4±0.03	0.3±0.01	0.93±0.02	0.49±0.01	0.53±0.01
Group II (*n*=41)	1.5±0.05	1.4±0.05	0.2±0.02	0.87±0.03	0.39±0.01	0.48±0.02
Group III (*n*=38)	1.4±0.04	1.4±0.06	0.2±0.02	0.91±0.05	0.43±0.02	0.48±0.02
P	I:II, III <0.05	>0.05	I:II, III <0.05	>0.05	>0.05	I: II, III <0.05

TFA=Total fibrinolytic activity; NFA=Nonenzymatic fibrinolytic activity; EFA=Enzymatic fibrinolytic activity; EBC=Exhaled breath condensate

Table 3: Diagnostic value of exhaled breath condensate indices concerning detection of a high risk of bronchial remodeling

EBC indices	Diagnostic value (95% CI)				LR	
	Test sensitivity	Test specificity	Prognostic value		PR	NR
			PR	NR		
Total protein content >4.0 g/L	52.6 (42.3-62.7)	58.3 (48.0-68.1)	55.8 (45.2-66.0)	55.2 (45.1-64.0)	1.3	0.8
AKDNPH of the main character >60.0 mmol/g of protein	31.2 (22.3-41.3)	73.3 (63.5-81.7)	53.9 (40.2-67.1)	51.6 (43.0-60.6)	1.2	0.9
Catalase activity >80.0 mcmol/min/mg of protein	28.6 (20.0-38.5)	86.7 (78.4-92.7)	68.3 (52.0-81.9)	54.8 (46.7-62.8)	2.2	0.8

AKDNPH=Aldehyde and ketone derivatives of 2,4-dinitrophenylhydrazone; CI=Confidence interval; LR=Likelihood ratio; PR=Positive result; NR=Negative result; EBC=Exhaled breath condensate

Table 4: Indices of high risk probability of bronchial remodeling according to the values of exhaled breath condensate markers

EBC indices	Risk ratio (95% CI)	Relative risk (95% CI)	Absolute risk
Total protein content >4.0 g/L	1.6 (0.9-2.7)	1.2 (0.9-1.7)	0.01
AKDNPH of the main character >60.0 mmol/g of protein	1.3 (0.7-2.3)	1.1 (0.7-1.7)	0.06
Catalase activity >80.0 mcmol/min/mg of protein	2.6 (1.3-5.4)	1.5 (0.8-2.7)	0.23

AKDNPH=Aldehyde and ketone derivatives of 2,4-dinitrophenylhydrazone; CI=Confidence interval; EBC=Exhaled breath condensate

frequent increased content of NMOM in EBC is found among the representatives of all the groups. Thus, the content of NO metabolites in EBC more than 40.0 mcmol/L in the Group I was found in 56.5% cases, in the Group II – in 58.8%, and in the group of patients with a low risk of bronchial remodeling – in 38.5% of observations (PI, II: III <0.05). It should be noted that diagnostic value as to the detection of patients with a high risk of bronchial remodeling according to the content of nitrogen monoxide metabolites in EBC more than 40.0 mcmol/L was insufficient: TS – 56.5% (95% CI 46.2–66.4), TS – 61.5% (95% CI 51.2–71.1), PVPR – 59.5% (95% CI 48.9–69.4), PVNR – 58.6% (95% CI 48.5–68.1); LR+ – 9.5%, LR (−) – 8.6%.

DISCUSSION

Thus, in children with "high risk" of respiratory tract remodeling more pronounced inflammatory process in the bronchi is found than in children with "low risk" which is evidenced by certain literary data. For example, Holgate,[8,9] who can be considered as one of the founder of inflammatory theory of disease, proved in the results of his experimental works that congenital or acquired inability of the epithelial barrier results in disorders of the epithelial-mesenchymal interaction, chronic inflammation, pro-inflammatory mediators secretion, increased sensitivity of the bronchial functional structures to them, and finally, to their remodeling. At the same time, in case of a repeated action of aggressive stimuli this inflammatory response acquires a chronic character. Herewith, disorders of a natural succession of the epithelial layer restoration processes occur, the processes of the epithelial cells migration are initiated under the influence of growth factors, secretion of extracellular matrix proteins and pro-inflammatory mediators take place.[10-12] It might be due to this fact that higher activity of proteolysis according to albuminolysis and azocololysis, higher indices of the condensate fibrinolytic activity, and more frequent detection of nitrogen monoxide metabolites content were found.

CONCLUSIONS

The results of EBC examination obtained enable to believe that in children with a "high risk" of respiratory tract remodeling more significant inflammatory process in the bronchi is found, than in children with a "low risk" which is proved by higher fibrinolytic and proteolytic activity, higher level of nitrogen monoxide metabolites, and it requires more aggressive basic anti-inflammatory therapy for these patients.

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Conflicts of interest

There are no conflicts of interest.

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