ORIGINAL ARTICLE

ABO blood group genotypes and ventilatory dysfunction in patients with allergic and nonallergic asthma

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ABSTRACT

Aim ABO blood group genotypes are established as a genetic factor in pathophysiology of various diseases, such as cardiovascular disorders, cancers, infectious diseases and there is rising evidence of their involvement in other conditions. The aim of this study was to determine if ventilatory changes of lung function in asthma, measured by biomarkers/parameters, are connected to certain ABO blood group genotypes in Croatia.

Methods A case-control study included 149 patients with asthma and 153 healthy individuals (blood donors). ABO genotyping on five main alleles was performed using PCR-SSP method. All patients had spirometry performed and severity of asthma was estimated. Clinical parameters of spirometry (FEV_1, FEV/FVC, PEF), biomarkers FeNO, IgE and pO2 were measured. The χ^2 test, Fisher's test, Kruskal-Wallis test and Spearman's correlation coefficients with $p<0.05$ were used as statistically significant.

Results There was no determined statistically significant difference in both ABO genotypes and phenotypes between patient and control groups. Comparison of the lung function in different ABO phenotypes in asthmatic patients also did not show any statistically significant differences in FEV1 values, FEV/FVC ratio or PEF. Statistically significant differences in oxygenation between different ABO blood types have not been noticed (p=0.326). Differences in quantitative values of biomarkers (FeNO and IgE) between different ABO blood phenotypes in patients with asthma were not significant, except for IgE that had marginal values (p=0.074).

Conclusion No correlation was found between certain ABO blood group genotypes and parameters/biomarkers of ventilatory dysfunction in patients with allergic and nonallergic asthma.

Key words: asthma, blood group antigens, respiratory function tests

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INTRODUCTION

The ABO blood group system is the most important one, because it represents an immunological barrier against transfusion of incompatible blood group or organ transplantation (1). Yamamoto cloned ABO genes in 1990 and that enabled more advanced ABO structural and functional analyses (2). This was followed by studies about ABO genes polymorphism and oligosaccharide antigens A and B, whose results are today successfully applied in forensics, transfusion, cell, tissue and organ transplantation and in cellular and evolutional biology (3). Years ago, the correlation between ABO blood group and incidence of certain diseases was established (4,5). That finding was followed by a series of studies that confirmed higher frequency of other tumours in blood type A phenotype (neurological, salivary glands, colon, uterus, ovarian, pancreas, kidney, urinary bladder) (6,7). In O blood type carriers, higher incidence of skin tumours and melanomas was detected (8). In the last decade research discovered a higher frequency of thromboembolic incidents in non-O blood type carriers and marked ABO genotypes as one of the genetic risk factors for cardiovascular diseases (9,10). There is a small number studies about the correlation of the ABO blood group system and incidence of various respiratory system diseases, such as rhinitis (11,12), asthma (13,14), tuberculosis (15), lung carcinoma (16) and the existing studies are based only on ABO phenotypes.

Asthma is an inflammatory disease characterized by bronchial hyper reactivity and periodical episodes of airway obstructions. It is one of the most common chronic diseases, especially in children, and it has a rising incidence in developed countries (17,18). Prevalence of asthma on the global scale today is 1-18%, depending on a geographical area (19). Asthma is the result of complex interaction between genetic and environmental factors and it is hard to define all the genes and biomarkers that are connected to pathogenesis of atopy and asthma. Previous studies showed some genes which have a great role in pathogenesis: IgE-receptor (FcεRI), cytokine genes and ADAM33 gene (20-22).

Defining asthmatic biomarkers and parameters, which is crucial for its diagnosis, and determining target spots in immunological reaction, would be an option to block the inflammation in asthma during specific immunotherapy (23). Clinical biomarkers FeNO, IgE and pO_2 are very important for patients suffering from asthma. The FEV1 (forced expiratory volume in first second) is the most used parameter of lung function, FEV1/FVC (forced vital capacity) ratio is a parameter of airway obstruction and PEF (peak expiratory volume) represents important parameter in the diagnosis and control of asthma (24).

There are contradictory results about ABO blood group genotypes as a genetic risk factor in pathophysiology of the asthma (13,14).

The aim of this study was to determine if ventilatory changes of lung function in asthma, measured by biomarkers/ parameters of disease, are connected with certain ABO blood group genotypes in Croatia.

PATIENTS AND METHODS

Patients and study design

This case-control study included 149 adult patients with asthma using medical records. The diagnosis of asthma was established through patients' history and the performance of clinical and laboratory tests. Blood samples were taken in the Clinical Department for Lung Diseases Jordanovac, the University Hospital Centre Zagreb between January and May 2017. A sample of 8.5 mL of blood was drawn in EDTA anticoagulant tube (Vacutainer PPT, Beckton Dickinson, USA). The blood samples were stored at -20°C until genomic DNA extraction.

A control group included 153 blood donors from Zagreb county, registered in the Croatian Institute of Transfusion Medicine (CITM) between January and May 2017; all were in good health, without any respiratory disease and with normal spirometry results.

All participants signed an informed consent. The study was approved by two Medical Ethics Committees: Clinical Department for Lung Diseases Jordanovac and CITM.

Methods

Clinical biomarkers/parameters measured in asthmatic patients. Spirometry was performed for all asthmatic patients. Ventilatory dysfunction was diagnosed according to spirometry results, and severity of asthma was estimated according

to recommendations of the Global Initiative for Asthma (GINA 2019) (19): intermittent asthma (GINA I), mild persistent asthma (GINA II), moderate persistent asthma (GINA III) and severe persistent asthma (GINA IV and V)(19). The following clinical parameters were also measured: serum level of IgE, partial pressure of oxygen in arterial blood (pO_2), FeNO in exhaled air, FEV1 value, FEV1/FVC ratio, and PEF.

ABO genotyping by PCR-SSP method (allele specific PCR). The samples of patients and blood donors were tested at the Department of Molecular Diagnostics of CITM using PCR-SSP (partially modified) method according to Gassner et al. (25). Five main ABO alleles were determined (O1, O2, A1, A1 and B) through eight parallel PCR-SSP reactions with coamplification of human growth hormone (HGH) gene fragment as positive internal control.

Statistical analysis

Descriptive statistics was used. Categorical and nominal variables were shown by absolute frequencies and corresponding portions, while quantitative values were shown by medians and interquartile ranges. Normality of data distribution was analysed by Kolmogorov-Smirnov test and based on the results of certain questionnaires; because of the small sample size, in further statistical analysis nonparametric statistical tests were used. The differences in categorical variables between patients and control group: ABO group was analysed using $\chi 2$ test, while Fisher's test was used in the analysis of statistical significance if there was at least one frequency in contingency table smaller than 5. The differences in quantitative variables were analysed using Kruskal–Wallis test. Spearman's rank correlation coefficients between certain quantitative values were calculated. The $p<0.05$ was considered significant.

RESULTS

This case-control study included 149 patients in the stable phase of asthma, 57 (38.3%) males and 92 (61.7%) females, with average age of 60 years. The control group included 153 healthy individuals, 71 (46.4%) males and 82 (53.6%) females, with average age of 43 years.

The leading genotype was O1O1, in 41 (27.5%) of the patients' group and in 46 (30.1%) cases of the control group. In blood type A phenotype group the most frequent genotype was O1A1 in both patients and control group, 34 (22.8%) and 39 (25.5%), respectively. In blood type B phenotype group the most frequent genotype was O1B1 in both patients and control group, 25 (16.8 %) and 27 (17.6%), respectively. In AB blood type carriers, A1B genotype was more frequent than A2B genotype in both groups, nine (6.0%) and three (2.0%) and 12 (7.8%) and three (2.0%), respectively. There was no statistically significant difference in the distribution of ABO genotypes and phenotypes between the patient and control groups (Table 1).

Table 1. Distribution of ABO phenotypes and genotypes in patients with asthma and in control group

ABO phenotype	ABO genotype	No (%) of samples		p
		Patients	Controls	
A				
	A1A1	4 (2.7)	6(3.9)	
	A1A2	3 (2.0)	1(0.7)	
	A2A2	1 (0.7)	0(0.0)	0.167
	01A1	34 (22.8)	39(25.5)	
	01A2	17 (11.4)	6(3.9)	
	02A1	2 (1.3)	2(1.3)	
B				
	01B	25 (16.8)	27(17.6)	
	02B	2 (1.3)	0(0.0)	0.135
	BB	0	2(1.3)	
AB				
	A1B	9 (6.0)	12(7.8)	0.756
	A2B	3 (2.0)	3(2.0)	
O				
	0101	41 (27.5)	46(30.1)	
	0102	8 (5.4)	8(5.2)	0.624
	0202	0	1(0.7)	
Total		149	153	

There were no statistically significant differences in FEV1 values (p=0.375), FEV/FVC ratio (p=0.741) or PEF (p=0.843) according to ABO phenotypes (Table 2).

Table 2. Comparison of lung function in different ABO phenotypes in 148 patients with asthma

Ventilatory capacity parameters	ABO phenotype	No of patients	Min.	Max.	Median	p
FEV1 (%)						
	O	49	34.80	125.30	68.00	
	A	60	20.90	119.40	80.10	0.375
	B	27	30.40	106.40	82.60	
	AB	12	36.00	101.30	66.25	
FEV1/FVC						
	O	49	37.69	99.26	65.47	
	A	60	38.08	98.82	68.79	0.741
	B	27	0.67	82.48	69.71	
	AB	12	40.26	83.24	68.40	
PEF						
	O	49	37.20	690.00	400.00	
	A	60	17.30	650.00	116.10	0.843
	B	27	30.80	650.00	395.00	
	AB	12	41.10	640.00	410.00	

Min., minimum; Max., maximum; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; PEF, peak expiratory flow

In 81 (54.4%) patients spirometry results did not show airway obstruction (FEV1 ≥80%), whereas in 68 (45.6%) patients obstructive ventilatory disorders were found (FEV<80%). Based on the spirometry, the severity of asthma was estimated as follows: 16 (10.7%) patients had intermittent asthma (GINA I), 27 (18.1%) patients had mild persistent asthma (GINA II), 45 (30.2%) had moderate persistent asthma, and 61 (40.9%) had severe persistent asthma.

Significant differences in oxygenation between different ABO blood types were not noticed (p=0.326) (Table 3).

Table 3. Differences in quantitative values of oxygenation between different ABO blood phenotype in 125 patients with asthma

Oxygenation	ABO phenotype	No of patients	Min.	Max.	Median	p
pO2 (kPa)	O	41	55.00	97.00	79.00	0.326
	A	52	52.00	95.00	79.00	
	B	23	60.00	99.00	78.00	
	AB	9	68.00	103.00	80.00	

Min., minimum; Max., maximum; pO2, partial pressure of oxygen in arterial blood

There were no statistically significant differences in aspect of both biomarkers, FeNO and IgE, although significance of IgE was marginal (p=0.074) (Table 4).

Table 4. Differences in quantitative values of FeNO and IgE biomarkers between different ABO blood phenotypes in patients with asthma*

Biomarkers	ABO phenotype	No of patients	Min.	Max.	Median	p
FENO	O	44	1.90	144.20	20.75	0.915
	A	55	4.30	134.00	19.00	
	B	26	3.50	112.50	18.55	
	AB	10	5.30	70.50	20.15	
IgE	O	41	1.00	5000.00	165.00	0.074
	A	47	6.28	2847.00	187.00	
	B	24	5.86	1418.00	108.50	
	AB	8	12.00	452.00	37.35	

*Correlation between biomarker FENO and ABO phenotypes were examined on 135 asthmatic patients and IgE on 120, respectively; Min., minimum; Max., maximum; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E;

DISCUSSION

We conducted this study primarily to investigate the correlation between ABO blood group system genotypes and ventilatory dysfunction in asthma, since only few studies investigated this relation (13,14), and this kind of research had not yet been conducted in Croatia. We found no statistically significant difference in the distribution of ABO phenotypes and genotypes between asthmatic patients and healthy controls as well as of lung function parameters between different ABO phenotypes, and quantitative values of FeNO.

De la Vega et al. found a higher frequency of blood type A in asthmatic children (26). Alo et al. came to a similar result, blood group A was significantly more frequent in asthmatic patients (37%) in comparison with healthy controls (26%) (27).

Brachtel et al. showed higher incidence of blood group antigens A and B in 239 German patients with atopic conditions (atopic dermatitis, hay fever, allergic rhinitis, bronchial asthma and acute urticaria), in comparison with 151 controls (28). Topno et al. conducted case-control study in Indian population that included 100 patients with symptoms of allergic rhinitis and 100 healthy controls, and found the most frequent blood group 0 among patients with allergic rhinitis (52%) (29).

The hypothesis that ABO system could be the one of genetic risk factors for the development of asthma is set by Ronchetti many years later (30), suggesting that ABO/secretor genes through their oligosaccharide structures (glycosyltransferases) control adhesion of infective agents. Thus, genetic variations in ABO blood group system could result in higher sensitivity to bacterial and viral infections and, because of that, could be an inductor of asthma development (30). Recent evidence suggests that asthma is associated with some kind of immunodeficiency responsible for an increased susceptibility to infection in asthmatic patients (31).

Although there are not many studies about the correlation between the ABO blood group genotypes and the development of asthma, it can be noticed that big variations in the study design, great heterogeneity of the results and final statistical analyses altogether make it difficult to come to some final conclusion (32). Interpretation of the results is difficult also due to genetic heterogeneity between various ethnic groups and local environmental exposure to allergens (33). The authors also emphasize inappropriate sample size, problems in the classification of asthma phenotypes or inadequate coverage of susceptible genes (34). There are even more published studies that investigate relationship of ABO blood group and asthma in asthmatic children than in adults (26, 30, 32). The limitation of our study is a small sample size of asthmatic patients and the fact that controls were not matched for age

and sex, but our study design of case–control, however, strengthens the evidence of the obtained results. The time-span of conducting the study is, by authors' opinion, not relevant, because ABO genotype is a genetically dependent variable, unchangeable through the passage of time.

Some results showed that carriers of blood type O/ secretor (SE/SE) and O/ LE (a-B-) were significantly associated with the development of asthma in childhood in Taiwan (35). Also, the older study of Kauffman et al. on the 228 adult coal miners showed significantly lower lung function and higher prevalence of wheezing and asthma in Lewis-negative, non-secretor, blood type O (13). A recent study in Brazilian patients with allergic rhinitis found significant difference in the incidence of carriers of O blood groups in males, but not females (11). It is an interesting result, because asthma and allergic rhinitis have similar immunopathology mechanism, and due to the fact that females more frequently suffer from asthma than males. Recently, Uwaezoke et al. critically review current evidence about linking ABO histo-blood group with the risk of respiratory atopy in children and adults published within the past 45 years (36). There are only eight studies taken in consideration and conclusions are that severe asthma is associated with B phenotype, while mild and moderate asthma is associated with O and A phenotypes (36). In contrast, the case-control study of Bijanzadeh et al. among 200 children and adults who suffered from bronchial asthma and 2000 controls in South India, showed no statistical correlation between the ABO system and development of asthma, which is exactly the same result as presented in our study (14). The authors from Malaysia, who analysed data from 14 studies about the ABO system and allergic diseases including asthma also emphasized a gap in geographic data and a need for further studies focusing on different populations (37).

Considering the given results and previous studies, further studies of correlation between the ABO blood group system and risk for asthma development are required, but with a much larger number of examined subjects and other genetic factors included, in order to confirm/reject the hypothesis that the ABO system could be one of the genetic risk factors for developing asthma.

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TRANSPERENCY DECLARATION

Competing interests: None to declare.

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