Original Article

Bronchial hyperresponsiveness to hypertonic saline challenge in children and adolescents*

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ABSTRACT

Objective: To assess airway hyperresponsiveness to 4.5% hypertonic saline solution in comparison to that obtained through challenge with other bronchoconstriction agents and in relation to patient allergic sensitization. **Methods:** A cross-sectional, experimental study was conducted, initially involving 85 subjects. After exclusions, the final sample consisted of 62 patients, divided into two groups: a study group of those with asthma (n = 45) and a control group of those with no asthma or allergies (n = 17). Hypertonic saline was nebulized using an ultrasonic nebulizer and administered successively for 0.5, 1, 2, 4 and 8 minutes until a drop in forced expiratory volume in one second of = 15% was achieved in relation to the baseline value. The level of specific immunoglobulin E to Dermatophagoides pteronyssinus level was determined by ImmunoCAP assay and was considered positive when > 0.35 kU/L. **Results:** In the 36 asthma group subjects presenting a response, the mean drop in forced expiratory volume in one second after hypertonic saline nebulization was 27.4%. None of control group subjects (immunoglobulin E < 0.35 kU/L) presented a positive response to hypertonic saline. The mean forced expiratory volume in one second for control group subjects was 9%. The results of a bronchial provocation test were negative in 9 of the asthma group subjects. The frequency of bronchial provocation test positivity was higher in the subjects presenting elevated levels of specific immunoglobulin E, indicating that there is a relationship between bronchial hyperresponsiveness and the level of specific immunoglobulin E. The sensitivity and specificity of the test were 80% and 92%, respectively. **Conclusion:** Bronchial provocation with hypertonic saline presents satisfactory sensitivity and specificity. Therefore, in addition to being a low cost procedure that requires very little equipment, it is a useful means of assessing hyperresponsiveness in children and adolescents.

Keywords: Bronchial provocation tests; Bronchial hyperreactivity; Saline solution, hypertonic; Administration, inhalation; Asthma; Child; Adolescent

INTRODUÇÃO

Asthma is the result of increased responsiveness of the physiological mechanisms that protect the airways, a condition known as bronchial hyperresponsiveness (BHR).[1]

Currently, there are various methods to quantify hyperresponsiveness. Among them, the most widely accepted, inexpensive and easily carried out is the pulmonary function test (spirometry), associated with the bronchial provocation (BP) method to induce bronchoconstriction. The BP tests are objective markers of asthma.[2] The gold standard method involves the use of pharmacological agents such as methacholine and histamine,[3] which act directly upon the receptors of the bronchial smooth muscle. Methacholine is difficult to obtain.[4] This, together with the fact that researchers continue to seek agents that mimic situations to which the respiratory system is subjected daily, has led to a search for alternative provoking agents. The first report on the use of hypertonic saline (HS) dates from 1981.[5] From that date on, there was great interest in substances that altered the osmolarity of the respiratory tract and were potent bronchoconstriction inducers.[6] Achieving BP by exercise and by HS inhalation are analogous methods, in which the most important stimulus is the hyperosmolarity of the fluid that covers the respiratory tract. During exercise, water is increasingly lost from the airways, moving toward the exterior due to physiological hyperpnea.[7] Inhalation of HS simulates this mechanism, with the advantage of being performed at tidal volume, therefore not requiring the cooperation of the patient to reach the maximum effort load. The increased osmolarity stimulates the release of mediators from the bronchial mucosa cells which, directly or indirectly, cause smooth muscle contraction.[6]

Although it can occur in normal asymptomatic individuals, BHR typically affects atopic individuals.[8]

The principal objective of this study was to evaluate inhalation of HS, a cheaper and more physiological alternative to other bronchoconstriction agents, comparing the spirometric response in normal individuals to that obtained in those with asthma and correlating it with the degree of allergic sensitization.

METHODS

The subjects with asthma were selected from specialized outpatient clinics, whereas the control group individuals were volunteers from public schools. The asthma group comprised males and females, ranging from 7 to 16 years of age. All had been diagnosed with asthma at least one year before the study outset and had a history of wheezing attacks within the preceding twelve months. On the day of the examination, any asthma group subject not presenting forced expiratory volume in one second (FEV_1) equal to or greater than 75% of predicted was excluded for fear that such individuals would present severe bronchoconstriction during the test. The asthma diagnosis and the classification of its severity were based on clinical (including allergy assessment) parameters and functional parameters, in accordance with the Brazilian Consensus on Asthma Management,[9] before the beginning of the treatment. The prophylactic medication was suspended prior to the examination: antihistamines for 48 hours, theophylline for 24 hours, 2-adrenergic for 24 hours and inhaled corticosteroids for 48 hours.[4]

The control group comprised males and females, from 7 to 16 years of age, with no chronic or nocturnal cough, no wheezing, no atopic dermatitis, no signs of allergic rhinitis, no history of bronchodilator use and no family history of asthma. All presented serum levels of specific immunoglobulin E (IgE) to Dermatophagoides pteronyssinus (Dp) 0.35 kU/L. During the study, some individuals in the control group presented high levels of specific IgE antibodies to Dp and were therefore excluded.

Patients having had a respiratory infection within the past four weeks were excluded.[10] All of the subjects followed the same protocol, approved by the Ethics in Human Research Committee of the Hospital de Clínicas of the Federal University of Paraná, and their legal guardians gave written informed consent.

We used an Ultra-neb 99 HD ultrasonic nebulizer (DeVilbiss, Somerset, PA) with expiratory valve and coiled hose. This is a high-volume nebulizer (240 mL) and, according to the manufacturer, it nebulizes 0.9% saline solution at an output rate of up to 6 mL/minute with aerosols presenting aerodynamic masses of less

than 4 m. The 4.5% HS was prepared in a class 100 - ll/B2 vertical laminar flow chamber and preserved in closed vials. Spirometry was performed using a Spirosift 3000 spirometer (Fukuda Denshi, Tokyo, Japan). The Polgar and Promadaht spirometric reference values were used.[11] A BG 1000 precision electronic scale (Gehaka, São Paulo, Brazil; minimum of 0.25 g, maximum of 1010 g, in 0.01 g increments) was used to weigh the metered-dose inhaler.

Blood samples were collected to measure serum levels of specific IgE to Dp using the CAP System method of fluoroenzyme immunoassay (UniCAP, Pharmacia Diagnostics, Uppsala, Sweden). The UniCAP test quantifies the level of sensitization by class: class 0 = < 0.35 kU/L; class 1 = 0.35-0.70 kU/L; class 2 = 0.70-3.50 kU/L; class 3 = 3.50-17.5 kU/L; class 4 = 17.5-50 kU/L; class 5 = 50-100 kU/L; class 6 = 100 kU/L. The individual was considered sensitized when IgE levels were 0.35 kU/L (class 1).[12]

The tests were performed in the morning, between 8:00 and 10:30 am, and the ambient temperature at the time of the test was determined and recorded. After the baseline FEV_1 had been registered, the BP procedure was initiated: HS inhalation rate = maximum; volume = 240 mL; weight = 750 g (metered-dose inhaler, HS and hose together). The metered-dose inhaler was weighed before and after each nebulization. It was then restored to its initial volume and weight with room temperature HS.[13-14] The participant remained in a sitting position, with a nasal clip, and was asked to breathe normally through the mouthpiece and to swallow any excess saliva. Elevation of the tube above the mouth and inversion of the mouthpiece with the expiratory valve upside down were necessary procedures to prevent the saliva from entering the nebulizer tube. The remainder of the solution in the tube, at the end of the inhalation, was re-included in the volume of the metered-dose inhaler and weighed thereafter. Each individual inhaled during 0.5, 1, 2, 4 and 8 minutes, totaling 15.5 minutes. The test was ended when there was a drop in FEV1 equal to or greater than 15%, or after a cumulative maximum of 15.5 minutes. The total substance inhaled was determined by the difference in weight after each nebulization. We decided that one gram worth of loss would correspond to 1 ml.[4] The spirometry was performed one minute after the end of the inhalation, with a two-minute maximum interval between nebulizations.[6]

The dose-response curve was defined using the semi-logarithm graphic, and registering the drop in FEV_1 in relation to the baseline after each inhalation and the cumulative inhaled volume at the same time. The HS volume that provoked a 15% drop in FEV_1 in relation to the baseline (provocative dose 15, PD15) was determined through interpolation. The responsiveness index was calculated through the ratio between the percentage of maximum FEV_1 reduction in relation to baseline and the total inhaled volume of HS.[4]

In the univariate analysis, the Student's t-test and analysis of variance (ANOVA) were used for continuous variables of normal distribution, and the Kruskal-Wallis test was used for continuous variables of asymmetric distribution. In order to evaluate the correlation among the categorical variables, we used the chi-square test and Fisher's exact test.

Logistic regression analysis was used to evaluate the probability of positive or negative BP, according to the responsiveness index.

RESULTS

A total of 85 children and adolescents were initially submitted to the protocol. After exclusions, a final sample of 62 patients completed the test: 45 in the asthma group and 17 in the control group (Table 1).

A total of of 23 individuals were excluded: 9 for presenting high levels of specific IgE antibodies to Dp; 6 for salt intolerance; 5 for lack of reproducibility of the expiratory maneuvers; and 3 for presenting baseline FEV_1 values lower than 75% of predicted.

Of the 45 subjects with asthma, 36 presented positive BP with a mean 27.4% drop in FEV_1 (range, 15-60%), mean variation, 4% (95% confidence interval) after HS inhalation. Of these 36 individuals with asthma, 26 (72%) still presented positive BP when a 20% drop in FEV_1 was used as the cutoff value. Nine individuals presented negative BP, with 14% maximal drop in FEV_1 (mean, 9%; range, 7-14%); mean variation, 1.7% (95% confidence interval), in the maximum cumulative inhalation period of 15.5 minutes. In the control group, maximal drop

TABLE 1

Clinical and anthropometric characteristics of the participants

Characteristic	Asthma (n = 45)	Control (n = 17)	p
Gender			
Male	24	4	0.005*
Female	21	13	
Age (years ± SD)	10.7 + 2.2	13.2 + 2.3	0.0001**
Height (cm ± SD)	146 + 0.13	158.3 + 0.11	0.0000**
Baseline FEV$_1$			
(% of predicted) ± SD	99.9 + 15.3	105.3 + 10.9	0.248**
IgE Dp (kU/L)	BP+(26) BP- (9)	BP - (17)	0.006***
< 0,35	1	2	17
> 0,35	1	0	
> 0,70	24	7	
Geometric mean	29.7	13.8	0.004***
Asthma severity	BP+(36)	BP- (9)	
Intermittent	8 (22%)	1 (11%)	1.00**
Mild persistent	13 (36%)	2 (22%)	
Persistent Moderate/Severe	15(42%)	6 (67%)	

The severity of asthma was classified, before treatment was initiated, according to the Brazilian Consensus on Asthma Management 2002. *Pearson chi-square test; **ANOVA; ***Fisher's exact test. SD: standard deviation; FEV$_1$: forced expiratory volume in one second; IgE: immunoglobulin E; BP+: positive bronchial provocation results; BP-: negative bronchial provocation results; Dp: *Dermatophagoides pteronyssinus.*

in FEV$_1$ after HS inhalation in the maximum cumulative period of 15.5 minutes was 13% (mean, 8.5%; range, 3-13%), mean variation, 1% (95% confidence interval). The difference between the asthma group and the control group in terms of the response to BP with HS was statistically significant (p < 0.05; Figure 1). All the participants presented cough at the beginning of the inhalation and sialorrhea during the test. However, as the experiment progressed, the patients adapted and the symptoms disappeared.

Of the asthma patients with a positive BP, 83% presented a drop of at least 15% in baseline FEV$_1$ at up to 4 minutes of inhalation. The nebulizer connected to the patient provided a mean output of 2.2 ml/min, with no difference between the groups. The mean PD15 of inhaled 4.5% HS in the asthma patients was 9.5 ml (standard error, 1.8 ml). The index of bronchial responsiveness correlated with the PD15 (p < 0.001, r = -0.98), and was significantly higher in the asthma patients. The participants with negative BP presented an index
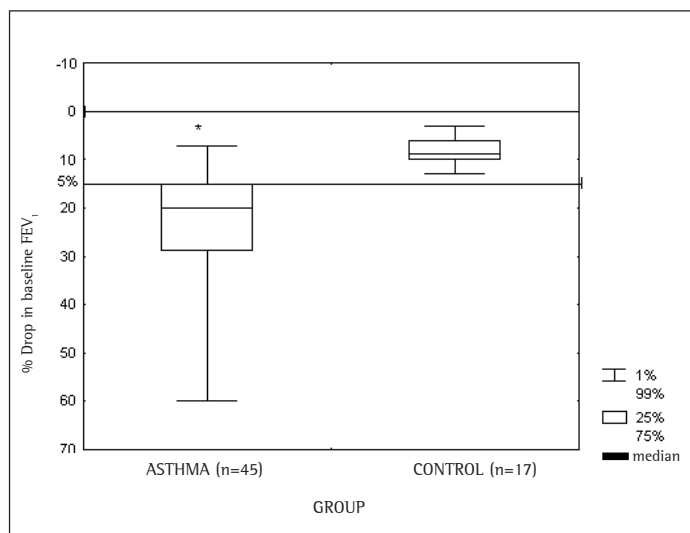

Figure 1 - Box plot of the percentage drops in FEV1 in relation to the baseline values; *p < 0.001 (Mann-Whitney)

lower than 0.33, and those with positive BP presented an index greater than 1.2, forming an overlap between 0.33 and 1.2, including BP-positive as well as BP-negative individuals. The logistic regression analysis demonstrated the probability of positive or negative BP according to the responsiveness indices.

The correlation of the serum levels of specific IgE to Dp in all the individuals who were submitted to BP (including those who were excluded due to a high specific IgE) revealed that these levels were
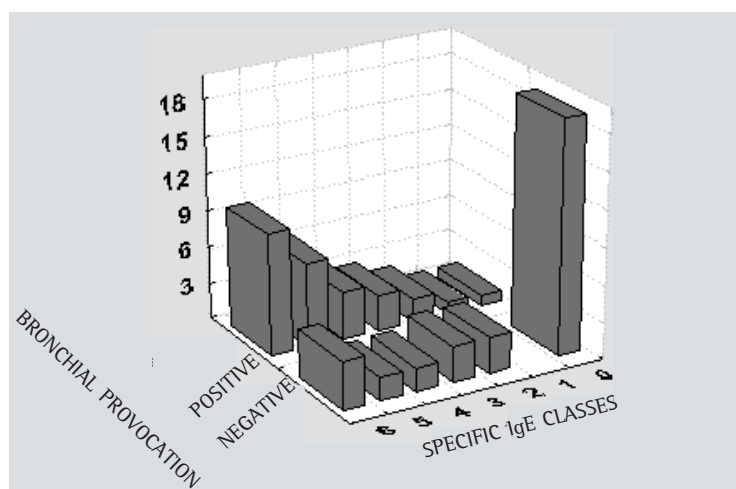

Figure 2 - Distribution of individuals presenting positive or negative responses to provocation in relation to the serum level class of specific IgE to *Dermatophagoides pteronyssinus*

higher in the individuals with positive BP. In addition, 93% of the individuals with positive PB presented levels greater than 0.70 kU/L, whereas only 42% of the individuals with negative PB presented the same levels (Table 1 and Figure 2). The higher the level of specific lgE to Dp, the greater the bronchial responsiveness to HS (Figure 2). Of the 9 patients who presented negative BP, 4 were already asymptomatic and were not receiving any treatment, but all of them had experienced asthma attacks within the preceding twelve months.

In all the individuals (n = 71) who were submitted to BP, regardless of their allergic status, the sensitivity and specificity of the test were 80% and 92%, respectively.

DISCUSSION

Inhalation of HS is useful to measure BHR, since it reproduces some conditions to which the respiratory epithelium is submitted, as is the case for physical exercise.[15] The 4.5% HS is slightly superior to the salt concentration in sea water.[16] The majority of the patients with asthma (80%) and some allergic individuals without asthma presented bronchial obstruction when submitted to the 4.5% HS inhalation. The nonatopic individuals presented no significant response to HS. The percentage drop in FEV_1 that determines hyperresponsiveness differs among various authors.[17-19] The value of 15% was used in this study because it is a value that increases the sensitivity of the method and reduces the risk of dyspnea and discomfort for patients with asthma.[20] The PD15 differentiates the groups with precision, since some individuals without asthma or allergies can present a drop of up to 10% when they inhale 0.9% HS.[21] In this HS inhalation study, control group subjects achieved a maximum FEV_1 drop of 10% (95% confidence interval).

Some authors[4] claim that there are no false-positive cases in BP by HS in healthy individuals, in contrast to the procedure with histamine and methacholine, in which up to 30% of the individuals present hyperresponsiveness to these substances, although they do not present any asthma symptoms. Although the histamine and methacholine doses are similar,[22] there is no correlation between the doses of methacholine and HS in the same individual. The excess of ions

is an additional factor in the response. Although 4% HS has the same osmolarity as does 6.1% dextrose, the former is significantly more potent that the latter.[6]

As children and adolescents grow older and taller, there is a decrease in bronchial responsiveness, probably due to maturation or to the relatively smaller dose of the bronchoconstriction medication, in relation to a bigger body.[23] In this study, even without the necessary adjustments for age and height, the results would not differ, since there is no information on the effect of these variables on the response to HS inhalation, as there is for the effect of methacholine.

The study demonstrated that the test is also useful in mild cases of asthma. Positive BP results were obtained for 5 participants with typical asthma symptoms but who were as then undiagnosed with the disease, as well as for 2 subjects with no asthma from the allergy group (without a personal or family history of asthma but with detectable specific lgE).

The comparison between the performance of the test in this study and in other studies should be carried out with caution, taking into consideration the differences in the inclusion and exclusion criteria, the severity of asthma, the type of nebulizer used and the output rates of the metered-dose inhalers.[24]

The family history of asthma and atopy, as well as the personal history of allergy, are risk factors for the development of BHR.[13] Regarding atopy, immunological changes include positive skin test response to allergens and high concentrations of circulating lgE.[25] The probability of developing clinical symptoms increases in parallel with the serum level of specific lgE antibodies, that could reach 100% when the levels are equal to or greater than 17.5 kU/L.[26] The sensitization to allergens varies depending on the region, although the most important antigens in positive skin tests or in the dosage of specific lgE in the solution are Dp and D. farinae,[27] the principal sensitizing agents for allergic airway disease of atopic subjects.[26] In this study, 93% of the patients with asthma were sensitized to Dp, which shows that testing for specific lgE antibodies to this allergen can be sufficient to identify most atopic individuals.[28-29]

There was a correlation between the serum

levels of specific IgE and the responsiveness index. However, this observation should be interpreted with caution, because when only the individuals with IgE > 0.35 kU/L are evaluated, there is no correlation of these levels with the index, or with the PD15. The magnitude of the sensitization influences the presence but not the intensity of the BHR. The specific antibody to Dp does not cause BHR, thereby indicating that other factors contribute to BHR in atopic individuals.

The BP response to HS is expressed in dose (PD15). This form has a negative correlation (r = -0.98) with the responsiveness index. This index correlates with the PD15. However, it is superior as an indicator of airway hyperresponsiveness since it considers the greatest drop in FEV_1 in relation to the total inhaled volume after the conclusion of the test.[30] The advantage of the responsiveness index over the BP is that the index can be used even if the PD15 is not reached. Another advantage is its practicality, since you only need to divide the percentage of the drop in FEV_1 by the total inhaled volume, whereas the PD15 requires a semi-logarithm graphic. A high responsiveness index indicates BHR.[30] According to the logistic regression analysis, the closer the index is to 0.33, the higher the chance of BP negativity, and the closer it is to 1.2, the higher the chance of BP positivity.

Asthma medication significantly decreases BHR, as well as controlling symptoms, reducing asthma severity, reducing HS sensitivity and even resulting in negative BP. The regular use of corticosteroids interferes with the results. However, due to ethical limitations, the inhaled corticosteroids were only suspended 48 hours before the test.[4] Nine individuals in the asthma group did not respond to BP with HS. This was probably a result of the treatment, since 45% of those patients were asymptomatic and were not under any medication at the time of the test. The correlation between BP results and asthma severity was not analyzed, since it referred to the pretreatment diagnosis. The principal limitations of this study, although not important, are worth mentioning, such as the unpleasant taste of salt in the mouth of the participant. The researchers solved this problem by asking that the participants drink water in the interval between inhalations. This problem was aggravated by the young age of the individuals and the prolonged period of time of the test, principally in the nonreactive patients.

The HS inhalation proved to be a useful BP method to evaluate BHR in children and adolescents, presenting adequate sensitivity and specificity.

This test is an inexpensive procedure that requires very little equipment and demands little cooperation from the patient.

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