Bronchoprovocation with hypertonic saline solution in asthmatic children

Broncoprovocação com solução salina hipertônica em crianças asmáticas

Brocoprovocación con solución salina hipertónica en niños asmáticos

Cínthia Maria X. Costa[1], Fernanda de Córdoba Lanza[2], Dirceu Solé[3]

ABSTRACT

Objective: To verify if the bronchoprovocation test with 4.5% hypertonic saline solution allows to detect the gradient of response in asthmatic children and adolescents, according to asthma severity.

Methods: 75 asthmatic patients aged six to 18 years-old were evaluated in this cross-sectional study. They were classified according to asthma severity in: intermittent or mild persistent (IM) and moderate or severe persistent (MS). They were also classified according to sensitization to inhaled allergens in atopics: positive skin prick test to *Dermatophagoides pteronyssinus*, *D. farinae* and *Blomia tropicalis*; or non- atopic with negative skin prick tests. All patients underwent a bronchoprovocation test with 4.5% hypertonic saline solution. The result of the bronchoprovocation test was considerd positive if at least a reduction of 20% in the forced expiratory volume in one second (FEV_1) was noted.

Results: 60 individuals were atopic. The bronchoprovocation test was positive more frequently in the MS group than in the IM one (93 *versus* 65%). Less time was needed for a 20% fall of FEV_1 in the MG compared to the IL group [90 (30–330) *versus* 210 (30–690) seconds; $p<0.05$]. The percentage of FEV_1 fall was higher in the MG group than in the IL one [26,4% (14–63) *versus* 20% (0–60); $p<0.05$].

Conclusions: The 4.5% hypertonic saline solution bronchoprovocation test is safe and easy to perform. It detects a gradient of response in asthmatic children and adolescents regarding asthma severity. Higher frequency of positive tests, shorter time for FEV_1 fall, and higher percentage of FEV_1 fall were observed in moderate and severe asthmatic patients.

Key-words: asthma; saline solution, hypertonic; spirometry.

RESUMO

Objetivo: Verificar se o teste de broncoprovocação, com solução salina hipertônica a 4,5%, permite detectar o gradiente de resposta em crianças e adolescentes com asma, segundo a gravidade da enfermidade.

Métodos: Estudo transversal composto por 75 pacientes asmáticos com idades entre seis e 18 anos. Os pacientes foram classificados pela gravidade (intermitente associada à persistente leve – IL, e persistente moderada associada à grave – MG) e segundo a presença de sensibilização a aeroalérgenos (testes cutâneos de hipersensibilidade imediata a *Dermatophagoides pteronyssinus*, *D. farinae* e *Blomia tropicalis*) ou não (atópicos *versus* não atópicos). Todos foram submetidos ao teste de broncoprovocação com solução salina hipertônica a 4,5%, considerando-se o resultado positivo se havia redução do volume expiratório forçado no primeiro segundo (VEF_1) ≥20%.

Resultados: 60 indivíduos eram atópicos. A frequência de positividade do teste de broncoprovocação foi maior no Grupo MG do que no IL (93 *versus* 65%). O tempo necessário para a queda de 20% do VEF_1 para o grupo de atópicos foi menor no MG quando comparado ao IL: 90 (30–330) *versus* 210 (30–690) segundos, com $p<0,05$. A porcentagem de queda do VEF_1 foi mais acentuada no subgrupo MG do que no IL [26,4% (14–63) *versus* 20% (0–60), $p<0,05$].

Conclusões: O teste de broncoprovocação com solução salina hipertônica a 4,5% é de fácil realização e seguro, permitindo detectar gradiente de resposta em crianças e adolescentes com

Instituição: Departamento de Pediatria da Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brasil

[1]Mestre em Pediatria e Ciências Aplicadas à Pediatria pela Unifesp; Fisioterapeuta; Docente do Centro Universitário de Ensino Superior de Maceió (Cesmac), Maceió, AL, Brasil

[2]Doutora em Pediatria e Ciências Aplicadas à Pediatria pela Unifesp; Fisioterapeuta; Docente da Universidade Nove de Julho (Uninove), São Paulo, SP, Brasil

[3]Professor Titular e Livre-Docente da Disciplina de Alergia, Reumatologia e Imunologia do Departamento de Pediatria da Escola Paulista de Medicina da Unifesp, São Paulo, SP, Brasil

Endereço para correspondência:
Cínthia Maria X. Costa
Loteamento Duas Irmãs, 89 – apto. 602 – Poço
CEP 57025-770 – Maceió/AL
E-mail: cinthiamcosta@hotmail.com

Conflito de interesses: nada a declarar

Recebido em: 28/7/2011
Aprovado em: 23/1/2012

asma segundo a gravidade da mesma. A maior frequência de positividade e a queda mais rápida do VEF_1 foram observadas nos pacientes com asma moderada ou grave.

Palavras-chave: asma; solução salina hipertônica; espirometria.

RESUMEN

Objetivo: Verificar si la prueba de broncoprovocación, con solución salina hipertónica a 4,5%, permite detectar el gradiente de respuesta en niños y adolescentes con asma, según la gravedad de la enfermedad.

Métodos: Estudio transversal, compuesto por 75 pacientes asmáticos, con edades entre seis y 18 años. Los pacientes fueron clasificados por la gravedad (intermitente asociada a persistente liviana - IL - y persistente moderada asociada a grave - MG) y según la presencia de sensibilización a aeroalérgenos (pruebas cutáneas de hipersensibilidad inmediata a Dermatophagoides pteronyssinus, D. farinae y Blomita tropicalis) o no (atópicos versus no atópicos). Todos fueron sometidos a la prueba de broncoprovocación con solución salina hipertónica a 4,5%, considerándose el resultado positivo como la reducción del volumen espiratorio forzado en el primer segundo (VEF1) ≥20%.

Resultados: Sesenta individuos eran atópicos. La frecuencia de positividad de la prueba de broncoprovocación fue mayor en el Grupo MG que en el IL (93 versus 65%). El tiempo necesario para la reducción de 20% del VEF1 para el grupo de atópicos fue menor en el MG cuando comparado al IL, 90 (30 a 330) versus 210 (30 a 690) segundos, con p<0,05. El porcentaje de reducción del VEF1 fue más acentuada en el subgrupo MG que en el IL, 26,4 (14 a 63%) versus 20% (0 a 60%), p<0,05.

Conclusiones: La prueba de broncoprovocación con solución salina hipertónica a 4,5% es de fácil realización y segura, permitiendo detectar el gradiente de respuesta en niños y adolescentes con asma, según la gravedad de la enfermedad. La mayor frecuencia de positividad y la reducción más rápida del VEF1 fueron observadas en los pacientes con asma moderada o grave.

Palabras clave: asma; solución salina hipertónica; espirometría.

Introduction

In Brazil, asthma is responsible for 350,000 hospitalizations annually, and it is the fourth leading cause of hospitalization in the Brazilian public Unified Health System (SUS), and the third among children and young adults. It is defined as a chronic inflammatory disease, characterized by hyperreactivity of the lower airways and airflow limitation, with spontaneous reversion or by treatment. It is classified regarding severity, according to clinic and spirometric parameters, in: intermittent, mild persistent, moderate persistent, and severe persistent[1].

The assessment of pulmonary function is the method of choice to determine the restriction of airflow and establishing the diagnosis of asthma. In symptomatic individuals with normal spirometry, the diagnosis of asthma can be confirmed by demonstrating the presence of bronchial hyperreactivity (BH) through bronchoprovocation tests (BPTs), which reflect the sensitivity or the ease of airway response to external stimuli or a marked response to β-2 agonist agents[1].

The BPTs considered gold standard are those using pharmacological agents such as histamine and methacholine[2-4]. However, the difficulty of access to these agents has motivated the search for alternative provocative agents. In 1981, there was the first report of the use of hypertonic saline solution (HSS)[4]. Inhalation on HSS increases water loss via airways to the outside world. After such osmolar stimulus, the release of mediators from bronchial mucosa cells occurs, causing the contraction of the smooth muscle and then bronchospasm[4-6].

BPT with HSS has good sensibility and specificity in the assessment of BH in children and adolescents. Although BH occurs in normal asymptomatic patients, patients with asthma have it more often[6-10]. In asthmatic patients, BPT with methacholine, although not universally, require gradient in the concentration of drug needed to induce the decrease of 20% or more in the forced expiratory volume in the first second (FEV_1) in relation to asthma severity. In most cases, more severe cases of asthma need lower doses of methacholine to induce the same level of bronchoconstriction[2].

In this context, the objective of this study was to verify if a BPT with HSS at 4.5% allows detecting gradient of response according to disease severity and the presence of atopy in asthmatic children and adolescents.

Method

This was a cross-sectional study, in which the sample size calculation was based on a pilot study with 14 individuals. These patients presented: intermittent asthma (three), with mean of 11.6 years and reduction of 20% of FEV_1 in mean time of 5 minutes (30 seconds to 9 minutes and 30 seconds);

persistent mild asthma (six), with mean of 8.1 years and mean time of decrease of 3 minutes and 54 seconds (1 minute and 30 seconds to 5 minutes and 30 seconds); moderate persistent asthma (three), with mean of 9.6 years and mean time of decrease in 2 minutes and 10 seconds (1 minute and 30 seconds to 3 minutes and 30 seconds); and severe persistent asthma (two), both with 11 years and mean time of decrease of 5 minutes and 30 seconds. Using as variable the time of FEV_1 decline (in seconds), considering the maximum error acceptable of 30 seconds, significance level of 5% and power of 80%, it would be necessary to include 75 patients. In this pilot study, it was found that children aged 6 or older had an adequate cognitive development to participate in the test.

The study included 75 patients aged between 6 and 18 years, diagnosed with asthma for at least 1 year and able to perform spirometry appropriately. These patients were referred by doctors from SUS outpatient services or from services linked to the Health Department of the state of Alagoas, which is a reference for the treatment and medical monitoring of children and adolescents with asthma.

Patients were non-smokers, had no other associated disease (pulmonary, cardiac or neurological) that could interfere in the evaluations nor had had upper respiratory tract infection in the last 30 days and/or exacerbation of asthma in the last week. In addition to clinical data obtained on admission, patients were classified according to severity of asthma in: intermittent, mild persistent, moderate persistent and severe persistent[1]. Then, patients and their parents were informed about the discontinuation of medications that could affect the test results: systemic corticosteroids (3 weeks), antihistamines and inhaled corticosteroids (48 hours), and theophylline and/or β-2 adrenergic agents (24 hours).

Patients underwent immediate hypersensitivity puncture skin tests to aeroallergens: *Dermatophagoides pteronyssinus, D. farinae* e *Blomia tropicalis* (FDA Allergenics®), taking the solution of histamine (1 mg/mL) as positive control and the excipient solution, as negative. We considered as a positive result the test that induced papular induration, whose diameter was higher than or equal to 3 mm[11]. Patients with at least one positive test were defined as atopic and those in which all results were negative, as non-atopic.

After acclimatization for 5 minutes in a special room at 22º C, children performed the measurement of their pulmonary function while seated, with nose clip and Easy One spirometer (pm) (Cradle Medical Technology®, model 2010, USA). All were previously trained for the proper execution of spirometry[12]. The values obtained (FEV_1, forced vital capacity – FVC – and the relation between FEV_1/FVC) were compared with normal values (Polgar and Promadhat[12]) and expressed in a percentage of the predicted value, being considered reference for the other evaluations.

In addition, vital signs were assessed: heart rate (HR) and respiratory rate (RR), axillary temperature (AT), blood pressure (BP) and pulmonary auscultation (PA). We measured O_2 peripheral saturation (SPO_2 – portable pulse oximeter, JG Moriya MI003®). During the evaluation the patients remained seated.

Patients were, then, submitted to the BPT with HSS at 4.5%, which is the most widely used because it combines high sensitivity, specificity, reproducibility and it is standardized[5], with an ultrasonic nebulizer (Pulmossonic Star Luxo II, Soniclear®) with 10 mL volume, flow rate around 1.25 mL/minute (steady), 80% aerosol and diameter less than 4 microns.

During the BPT, children inhaled HSS at 4.5% for increasing periods (0.5; 1; 2; 4 and 8 minutes) at intervals of 2 minutes, totaling 15 minutes and 30 seconds, depending on a positive response or not. FEV_1 was measured 1 minute after the end of each inhalation. Drops of FEV_1 equal or higher than 20% in relation to the basal value authorized the interruption of BPT. Patients who did not have a drop after 15 minutes and 30 seconds of inhalation were categorized as having negative bronchoprovocation[4,5].

At the end of BPT, a new evaluation was performed: vital signs, PA and SpO_2. Patients also received inhalation with β-2 agonist agent of short duration (salbutamol aerosol via metered dose inhaler – 400 mcg/dose) to relieve possible bronchospasm.

For statistical analysis, initially, we performed the comparison between the different degrees of severity of asthma. The absence of differences made the patients with intermittent and persistent mild asthma comprise the IM Group while those with persistent moderate or severe asthma composed the MS Group. The IM Group worked as a control group, once the Research Ethics Committee of Universidade de Ciências da Saúde de Alagoas (Uncisal) did not authorize the submission of non-asthmatic individuals to BPT with HSS at 4.5%. All variables were also analyzed considering atopic patients versus non-atopic ones.

Sample homogeneity was verified by the Kolmogorov-Smirnov test. The variables analyzed did not present normal distribution, and their values were described as median values. The Kruskal-Wallis non-paramedic test was employed for variables such as time and percentage of FEV_1 decrease, according to severity of asthma and the presence of atopic status. For the analysis of vital signs changes, we used the Mann-Whitney non-paramedic test. We considered as statistical significance when $p \leq 0.05$.

The study was approved by the Research Ethics of Universidade Federal de São Paulo (Unifesp), protocol 1039/08, and Universidade de Ciências da Saúde de Alagoas (Uncisal), protocol 817/2008. Patients and their guardians were informed about the research and signed an informed consent form.

Results

Out of all asthmatic patients referred to be submitted to BPT with HSS at 4.5% (n=96), eight patients were not admitted for having basal value of FEV_1 70% lower than the predicted value, other eight because they presented other associated diseases that could interfere with the evaluations and five because they could not perform acceptable and reproducible spirometry. Thus, 75 children and adolescents with asthma were included in the study.

Such patients, after the immediate hypersensitivity skin test, composed two groups: atopic (n=60) and non atopic (n=15) to household dust mites (Table 1). At the beginning of the outbreak, the two groups were similar in relation to age and predicted basal values of FEV_1 (Table 1). BPT with HSS was positive in 58 patients (77.3%), 9 (60.0%) non-atopic and 48 (80.0%), atopic. Although there was no difference between the percentage values of FEV_1 at the end of the test, the time needed for the drop to occur was significantly higher in the non-atopic. The percentage of FEV_1 reduction in relation to basal values was higher among the atopic, when compared to non-atopic patients (Figures 1 and 2). Considering individuals with negative test, the mean reduction of the FEV_1 was 2.6% (variation of <1 to 5%) among the non-atopic and 10.7% (variation of <1 to 17%) among atopics. Among the atopic patients, four patients (30%) presented reduction higher than 15%.

The initial analysis, considering the severity of asthma as variable, did not show statistically significant differences between patients with intermittent and mild persistent asthma, what allowed us to group them (intermittent + mild – IM Group). The same occurred in relation to patients with moderate persistent asthma and severe persistent asthma (moderate group + severe – MS). However, among the non-atopics, the

Table 1 - Clinical and anthropometric characteristics of children (described as median, higher and lower limits)

	Atopic	Non-atopic	*p*-value
Gender (M/F)	38/22	4/11	
Age (years)€	10 (6 to 15)	11 (6 to 15)	0.273
6 to 7 years	12	04	
8 to 10 years	23	01	
≥11 years	25	10	
BMI	17.1 (13 to 26)	18(13 to 26)	0.904
Initial FEV_1 (% predicted)*	80.9 (70 to 119)	81.1 (70 to 117)	0.900
After FEV_1 (% predicted)*	59.7 (28 to 91)	68.1 (41 to 114)	0.196
Skin prick test positive for:			
D. pteronyssinus (%)	53 (88.3)	0	
D. farinae (%)	37 (61.6)	0	
Blomia tropicalis (%)	56 (93.3)	0	
Asthma Severity*	**BPT+vo (% BPT+vo)**	**n**	
Intermittent	15	8	
	11 (73.3)	4 (50.0)	
Mild persistent	21	2	
	14 (66.6)	1 (50.0)	
Moderate persistent	16	5	
	15 (93.7)	4 (80.0)	
Severe persistent	8	0	
	8 (100.0)	(0)	

FEV_1: forced expiratory volume in fist second; *D. Dermatophagoides*; BPT+vo: positive bronchoprovocation test; %BPT+vo: percentage of positive bronchoprovocation in relation to the group; *according to IV Brazilian Guidelines on Asthma Management 2006[1], before starting treatment; n.: number of individuals per group

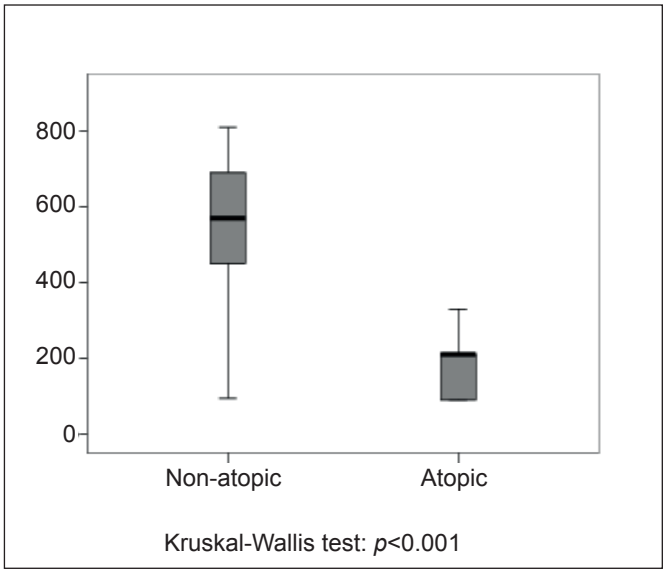

Figure 1 - Time needed (seconds) for 20% reduction in FEV_1, in the group of children and adolescents with asthma, according to presence of atopy

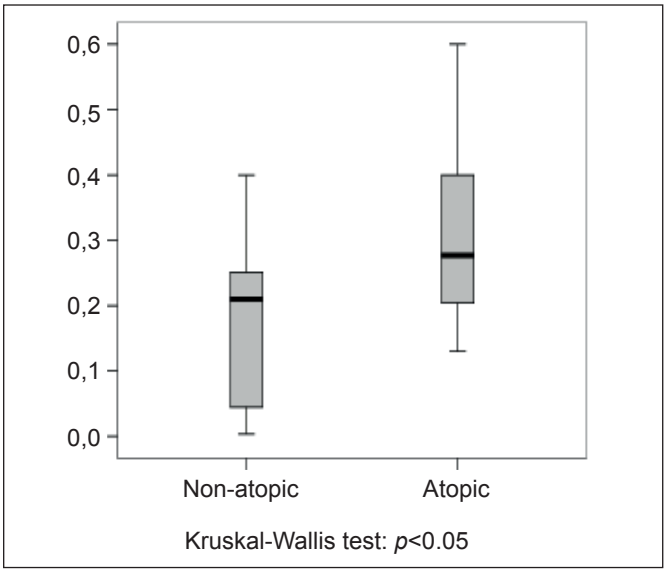

Figure 2 - Reduction in FEV1 (percentage) in children and adolescents with asthma, according to presence of atopy

comparison between patients with persistent moderate asthma and patients with persistent severe asthma was not possible, due to the fact that there were no patients with severe persistent asthma in this group. Among atopic and non-atopic patients, there were no significant differences regarding predicted basal values for the pulmonary parameters assessed according to asthma severity. Among the atopics, we verified significantly lower values in the ratio FEV_1/FVC and in the peak of expiratory flux (PEF) in the MS patients after BPT with HSS, a fact that was not observed among non-atopics. Regarding the FEV_1, a significant reduction was observed among those with M asthma in the non-atopic group. In the atopic group, there was a reduction on the time needed for the 20% drop of FEV_1 to occur and higher percentage of drop in predicted FEV_1 among those with MS asthma after BPT, which was not observed among the non atopic.

The comparative analysis of the presence or absence of atopy among patients, divided according severity of asthma, showed significant changes only in Forced Expiratory Flow from 25 to 75% (FEF_{25-75}) in the IM Group, before and after bronchoprovocation, and it was higher among non-atopics. Variation of FVC was lower than 5% after bronchoprovocation. Comparison between MS subgroups showed that the predicted value of FEV_1 after bronchoprovocation was lower in the atopics, as was the time needed for the 20% drop in FEV_1. The presence of asthma induced by exercise was not evaluated.

Vital signs represented by RR, HR, systolic BP (SBP), diastolic BP (DBP), AT and SpO_2, both before and after bronchoprovocation, were similar between groups. Among atopics, there was an increase in HR and a decrease in SpO_2, after bronchoprovocation in the IM subgroup. In this group there were no changes in other vital signs after the exam. In the MS subgroup, only the SpO_2 decreased. In the non-atopic group, there were no differences in vital signs before and after bronchoprovocation between subgroups.

Discussion

The BPT with saline solution proved to be safe, since its interruption was not necessary in any patient because of the emergence of adverse effects, by request of the patients or because they felt bad or uncomfortable during the procedure.

In this study, BPT with HSS at 4.5% documented the presence of BH in 77% of patients, similar data to those observed by other researchers (between 48.9 and 80%)[4-6,12,13]. However, when considering the distinction between atopic and non-atopic, this rate reaches 80%, corroborating with other studies [4,5].

The presence of atopy is associated with increased BH, possibly because of inflammatory changes triggered by repeated exposure to the allergen. On the other hand, the resulting BH causes increased inflammation response and symptoms[1]. In the present study, patients that were sensitive to dust mites were considered atopic, because the latter are the main etiologic agents of respiratory allergies in tropical areas[14].

The frequency of positive BPT with HSS at 4.5% was higher in patients with moderate and severe asthma, who remained more symptomatic. A similar finding was observed by other authors, who reinforced the existence of a direct relation between the number of wheezing episodes and de degree of BH[15,16].

The presence of gradient response to BPT, with HSS at 4.5%, depending on the severity of asthma was also documented. Although the two groups of patients were initially similar to FEV_1, when submitted to BPT with HSS at 4.5%, those atopic with persistent or severe asthma presented a faster and more marked drop in FEV_1 than the group with intermittent or mild persistent asthma. In a way, these findings support the idea that individuals with more severe asthma have more pronounced BH. Other studies have observed the relation between severity and bronchial reactivity by different methods. Cassol *et al* found more profound decline in FEV_1 in individuals with severe asthma, followed by those with moderate and severe, with exercise induced methods[16]. Avital *et al* performed BPT with methacholine in children with mild or moderate asthma and confirmed the need for lower doses of methacholine to determine the drop of at least 20% in FEV_1 values, among those with moderate asthma[17].

Another point to consider regarding the parameter used for the diagnosis of BPT as positive: 20% drop in baseline[5] FEV_1 or 15% drop[4,6], which alters the sensibility to BPT. Because of this, we fixed the positivity rate for BPT with HSS at 4.5% in 20%.

The measurement of FVC may vary depending on bronchial obstruction, elastic recoil, and muscular effort; the PEF reflects the proximal airways caliber and is a dependent effort[12]. Thus, reduction of these values could mean bronchial obstruction caused by the test, as it could also suffer interference from the fatigue caused by the various spirometric tests performed. The median of FVC was reduced, in the IM Group, in 8.6% for non-atopic and 15.8% for atopic, and, in the MS Group, the reduction was of 1.4% for non-atopic and 18.6% for atopic. There was also a reduction in PEF, in which the median for the IM Group was of 19.6 (non-atopic) and 22.6% (atopic), as well as for the MS Group, 23.5 (non atopic) and 31.8% (atopic).

The FEF_{25-75} is considered the most sensitive parameter for the detection of obstruction in the populations at risk, and represents the changes in the peripheral airways of medium and small caliber[12,18]. The reduction of this parameter was marked, taking as reference studies that considered as positive response the decrease in FEV_1 and/or in the FEF_{25-75} of at least 25%[19]. In the present study, we observed a decrease in the median of the IM Group of 27.4% (non-atopics) and 34.5% (atopics), as well as in MS, of 36.1% (non-atopics) and 44.0% (atopics). As the variation of FVC was lower than 5% after bronchoprovocation, the alterations of FEF_{25-75} may have resulted from the inhalation.

Considering the other clinical variables analyzed, there were no significant changes, except for the increase in HR in the IM atopic group. Even with this change, patients remained within normal limits, without health risks. The SpO_2 showed a slight decrease in the atopic group, but the reduction, although statistically significant, apparently had no clinical significance, which was reduced from 98 to 97%, and the decline was more pronounced among those characterized as atopic. A study performed by Solé *et al* found no relationship between the decrease in SpO_2 and provocative concentration, and found no relation between the decrease in SpO_2 and the percentage of FEV_1 variation in BPT[20].

BPT with HSS at 4.5% proved to be safe, easy to perform and required little cooperation from participants. At the end, most patients complained about the unpleasant taste of the salt, the excessive production of saliva, the prolonged duration of the test, particularly among those in which there was no induction, besides fatigue for the repeated spirometry calculations, especially in younger individuals.

In the present study, it was verified that BPT with HSS at 4.5% determines the gradient of answer in children and adolescents with asthma, according to the severity of the disease. Severe forms are followed by greater and faster drops in FEV_1. The frequency of positive BPTs was higher among atopic patients, as well as in those with more severe asthma. According to some authors[1,2], the documentation of the presence of BH has been essential for the diagnosis of asthma, especially in patients with atypical forms. Thus, a BPT with SSF at 4.5%, which is safe, easy to perform, and allows documenting gradient intensity of asthma, should be an important instrument to be incorporated in the follow-up of patients with asthma, because it allows an objective assessment of the disease.

Aknowledgements

To Centro Universitário Cesmac for the support, to the physiotherapists Ana Lúcia de Gusmão Freire, Patrícia Nobre, Edjane de Biase and Jaciene Farias. To the doctors who cooperated in this research: Dirlene Brandão, Rosa Maranhão, Ana Mary Xavier and Ana Cláudia B. Dawsley. To nurse Rita and Doctor Rogério Barboza.

References

1. Stirbulov R, Bernd LA, Solé D. IV Diretrizes brasileiras para o manejo da asma. Rev Bras Alerg Imunopatol 2006;29:222-45.
2. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG *et al*. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 2000;161:309-29.
3. Matos FL, Terra Filho J, Martinez JA, Sala TF, Vianna EO. Airway inflammation in asthmatic patients after methacholine challenge. J Pneumol 2001;27:171-6.
4. Kussek P, Rosario Filho NA, Cat M. Bronchial hyperresponsiveness to hypertonic saline challenge in children and adolescents. J Bras Pneumol 2006;32:195-201.
5. Smith CM, Anderson SD. Inhalation provocation tests using nonisotonic aerosols. J Allergy Clin Immunol 1989;84:781-90.
6. Riedler J, Reade T, Robertson CF. Repeatability of response to hypertonic saline aerosol in children with mild to severe asthma. Pediatr Pulmonol 1994;18:330-6.
7. Cockcroft DW. Direct challenge tests: airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest 2010;138 (Suppl 2):18S-24.
8. Rullo VE, Rizzo MC, Solé D, Naspitz CK. Broncoprovocação específica em crianças asmáticas. Rev Bras Alergia Imunopatol 1996;19:263-71.
9. Solé D, Mello Júnior JF, Weckx LL, Rosário Filho NA. II Consenso Brasileiro sobre Rinites 2006. Rev Bras Alerg Imunopatol 2006;29:29-58.
10. Melo RM, Lima LS, Sarinho ES. Relationship between home environmental control and exacerbation of asthma in children and adolescents in the city of Camaragibe in the state of Pernambuco, Brazil. J Bras Pneumol 2005;31:5-12.
11. Forte WC, Júnior FF, Filho WD, Shibata E, Henriques LS, Mastroti RA *et al*. Positive skin test and age. J Pediatr (Rio J) 2001;77:112-8.
12. Pereira CA, Lemle A, Algranti E, Jansen JM, Valença LM, Nery LE *et al*. I Consenso brasileiro sobre espirometria. J Pneumol 1996;22:98-164.
13. Choi IS, Chung SW, Koh YI, Sim MK, Hong SN, Moon JS. Airway hiperresponsiveness to hypertonic saline as a predictive index of exercise-induced bronchoconstriction. Korean J Intern Med 2005;20:284-9.
14. Rizzo MC, Solé D, Rizzo A, Holanda MA, Rios JB, Wandalsen NF *et al*. Atopic diseases in Brazilian children–etiologic multicentric study. J Pediatr (Rio J) 1995;71:31-5.
15. Mai XM, Nilsson L, Kjellman NI, Björkstén B. Hypertonic saline challenge tests in the diagnosis of bronchial hyperresponsiveness and asthma in children. Pediatr Allergy Immunol 2002;13:361-7.
16. Cassol VE, Trevisan ME, Moraes EZ, Portela LO, Barreto SS. Exercise-induced bronchospasm in children and adolescents with a diagnosis of asthma. J Bras Pneumol 2004;30:102-8.
17. Avital A, Godfrey S, Springer C. Exercise, methacholine, and adenosine 5'-monophosphate challenges in children with asthma: relation to severity of the disease. Pediatr Pulmonol 2000;30:207-14.
18. Pereira CA, Barreto SP, Simões JG, Pereira FW, Gerstler JG, Nakatani J. Valores de referência para a espirometria em uma amostra da população brasileira adulta. J Pneumol 1992;18:10-22.
19. Rodrigues JC, Takahashi A, Olmos FM, Souza JB, Bussamra MH, Cardieri JM. Effect of body mass index on asthma severity and exercise-induced bronchial reactivity in overweight and obese asthmatic children. Rev Paul Pediatr 2007;25:207-13.
20. Solé D, Gomes IC, Vanna AT, Guelpa A, Mallozi MC, Naspitz CK. Oximetria de pulso na avaliação da broncoprovocação com metalina e por exercício em crianças com asma. Rev Bras Alergia Imunopatol 1998;21:46-51.