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LUCAS PINTO SALLES DIAS

OSCILOMETRIA DE IMPULSO, REMODELAMENTO E MEDIADORES INFLAMATÓRIOS E FIBRÓTICOS EM CRIANÇAS ASMÁTICAS

IMPULSE OSCILLOMETRY, REMODELING AND INFLAMMATORY AND FIBROTIC MEDIATORS IN ASTHMATIC CHILDREN

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Orientador: Prof. Dr. Rodolfo de Paula Vieira

Artigo apresentado ao Programa de Pós-Graduação em Bioengenharia da Universidade Brasil, como complementação de créditos necessários par à obtenção do título de Mestre em Bioengenharia.

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SUMÁRIO

1. INTRODUÇÃO

Considerada uma doença heterogênea e usualmente definida por uma inflamação crônica das vias aéreas, que leva a uma limitação variável ao fluxo aéreo, caracterizada pela história de sintomas clássicos como sibilância, respiração curta, aperto no peito e tosse que variam em tempo e intensidade. (Global Initiative for Asthma - GINA 2017). A asma afeta mais de 334 milhões de pessoas mundialmente e 14% das crianças em todo mundo já sofreram algum sintoma da asma, segundo dados de 2014 da organização mundial de saúde (OMS). (Global Initiative for Asthma - GINA 2017).

A espirometria é o método de escolha na avaliação da limitação ao fluxo aéreo e no diagnóstico da asma, as medidas comumente utilizadas são o volume expiratório final no primeiro segundo (VEF_1), relação VEF_1 pela CVF e o fluxo expiratório forçado entre 25-75% da capacidade vital ($FEF_{25-75\%}$) (IV Diretriz Brasileira para Manejo da Asma, 2006 ; Manoharan et. Al., 2015**)** São indicativos de asma valores de VEF1 inferior a 80% do previsto, relação VEF1/CVF inferior a 75% em adultos e 86% em crianças ou obstrução ao fluxo aéreo que desaparece ou melhora significativamente após a administração de broncodilatador. (Global Initiative for Asthma - GINA 2017 ; III consenso brasileiro para manejo da Asma, 2002).

O diagnóstico confiável de asma em criança é difícil, visto que os sintomas clássicos da enfermidade são comuns em menores de 2 anos e a avaliação da limitação do fluxo aéreo é dificultada pois requer colaboração do indivíduo, o diagnóstico então é baseado nos sintomas, associado à avaliação clínica minuciosa e achados físicos (Global Initiative for Asthma - GINA 2017).

Tecnologias modernas vêm sendo desenvolvidas para avaliação da função pulmonar, porém não são aplicadas rotineiramente em sujeitos com desordens intelectuais e crianças jovens, levando a diagnósticos e tratamentos inadequados. Diversos estudos têm demonstrado a oscilometria de impulso- *Impulse oscillometry system* (IOS) é uma ferramenta útil e confiável para diagnóstico da asma em crianças devido à sua facilidade de aplicação, necessitando de apenas uma pequena colaboração do indivíduo avaliado em manter o volume corrente respiratório. (Vink et. al., 2003 ; Song et. al., 2008 ; Marotta et. al., 2003 ; Desenvolvida em 1956 por Dubois et. al. a partir da teoria das vibrações, surgiu como modificação da técnica de oscilações forçadas (TOF) que se difere basicamente por medir resistência (R) e reatância (X) pulmonar em várias faixas de frequência diferentes, permitindo uma análise útil da variação da impedância (Z) respiratória e também da frequência de ressonância (Fres) (Assumpção et. al., 2014). Os parâmetros oscilométricos são expressos em frequências distintas: 5, 10, 15, 20, 25, 30 e 35 Hertz (Hz), o componente R reflete principalmente a perda da fricção que ocorre durante o fluxo de ar nos brônquios, enquanto o X contempla a energia armazenada nos componentes mais periféricos do sistema respiratório (Assumpção et. al., 2014). Marotta et. al. comparou a IOS com a espirometria convencional na resposta ao broncodilatador em crianças de 4 anos com propensão à asma e encontrou alterações de resistência a 5Hz (R5) e resistência em 10Hz (R10) somente nas crianças asmáticas (Marotta et. al., 2003), corroborando com os dados encontrados no estudo de Song et. al. onde foi identificado alterações em R5, R10, R20 e R35 em crianças coreanas asmáticas de 3 a 6 anos, em comparação com crianças hígidas, em relação a resposta ao broncodilatador (Song et. al., 2008).

A inflamação das vias aéreas é uma anormalidade dominante no asmático que é resultado de um amplo e complexo espectro de interações entre células inflamatórias, mediadores e células estruturais das vias aéreas (IV Diretriz Brasileira para Manejo da Asma, 2006). Essa resposta ocorre mediante à vários estímulos como alérgenos, poluentes ou infecção viral, que ativam células da mucosa brônquica que liberam mediadores inflamatórios, causando infiltração eosinofílica, sendo o eosinófilo um marcador importante da inflamação na asma. (Barning et. Al., 2018). Através dos mastócitos são liberados: histamina, leucotrienos, triptases e prostaglandinas; pelos macrófagos: fator de necrose tumoral alfal (TNF-alfa), interleucina 6 (IL-6) e óxido nítrico (NO); por meio dos linfócitos T: interleucina 2 (IL-2), interleucina 3 (IL-3), interleucina 4 (IL-4), interleucina 5 (IL-5); pelos neutrófilos a elastase e através das células epiteliais; endotelina-1, mediadores lipídicos e NO. (IV Diretriz Brasileira para Manejo da Asma, 2006). Através dos mediadores da inflamação ocorrem lesões e alterações na integridade do epitélio respiratório, anormalidades no controle autonômico e no tônus da via aérea, hipersecreção de muco e aumento da reatividade do musculo liso da via aérea (hiper-reatividade brônquica) (IV Diretriz Brasileira para Manejo da Asma, 2006).

O óxido nítrico (NO) é o biomarcador não invasivo mais amplamente utilizado na análise da inflamação das vias aéreas, no trato respiratório ele é produzido por vários tipos de células (células epiteliais, nervos da via aérea, células inflamatórias e do endotélio vascular), uma concentração elevada de NO é observada em asmáticos quando comparado com indivíduos saudáveis (Muñoz et. al., 2015). Um nível maior que 50 partes por bilhão (ppb) auxilia no diagnóstico de asma eosinofílica em adultos sintomáticos, além de predizer a

resposta ao tratamento com corticoesteróides e um nível alto e persistente de óxido nítrico exalatório (eNO) sugere não adesão ao tratamento ou uma técnica inalatória inadequada (Loutsios et. al., 2014). De acordo com a recomendação da American Thoracic Society (ATS) e European Respiratory Society (ERS), o eNO apresenta-se como um marcador útil no diagnóstico da asma, para monitorar a resposta à terapia anti-inflamatória, verificar aderência à terapia e predizer a exacerbação da asma (American Thoracic Society, 2005)

A coleta do ar condensado é uma técnica não invasiva, onde o ar exalado é congelado e há fortes evidências que anormalidades na composição do ar condensado refletem alterações bioquímicas no fluido das vias aéreas (Kharitonov et. al., 2006). Leucotrienos, prostaglandinas, lipoxinas e citocinas são marcadores específicos da inflamação e podem ser mensurados na análise do ar condensado, cis-leucotrienos, LTB4, IL-4, IL-5, IL-8 e IL-17, TNF-alfa e TGF-beta estão todos aumentados no ar condensado de pacientes asmáticos quando comparados com pessoas saudáveis (Muñoz et. al., 2015). O pontencial de hidrogênio (PH) também pode ser avaliado no condensado exalado, onde em asma aguda este apresenta grande diminuição (Kharitonov et. al., 2006). Um aumento dos níveis de leucotrienos no ar condensado está correlacionado com a severidade da asma, e a literatura demonstra que em pacientes asmáticos é encontrado aumento da IL-4, IL-5, IL-6, IL-8, IL-10 e IL-17 na análise do condensado (Konstantinidi et. al., 2015).

A análise dos biomarcardores séricos é importante para avaliação da inflamação e endotipos e fenótipos de asmáticos, a análise dos eosinófilos mostra que ele está, na maioria dos casos, aumentado na asma, tanto em crianças quanto em adultos é observada correlação entre a contagem de

eosinófilos e os sintomas, e uma correlação inversa em relação ao $VEF_{1,}$ estes são responsáveis por produzirem mediadores pró-inflamatórios nos tecidos levando a danos e lesões (Szefler et. al., 2012). A IL-4 e IL-13 apresentam um papel importante no tráfego de eosinófilos, enquanto a IL-5 exerce papel fundamental na proliferação, diferenciação e ativação dos eosinófilos (Szefler et. al., 2012).

A IL-8 está correlacionada com a extensão da inflamação neutrofílica e com a severidade da doença, IL-6 está significativamente alta em pacientes com asma neutrofílica e somando-se com IL-17 e TNF-alfa são consideradas citocinas pró-inflamatórias indiretamente relacionadas à inflamação sistêmica durante a exacerbação da asma (Rufo et. al., 2013). A maioria das pesquisas em asma está focada na asma alérgica, este fenótipo é caracterizado por presença de células Th2 específicas para o alérgeno, produzindo citocinas como IL-4, IL-5, IL-9 e IL-13, associado a aumento da imunoglobulina E (IgE) específica e também aumento dos eosinófilos séricos (Rufo et. al., 2013).

As citocinas IL-4, IL-5, IL-10 e IL-13, são os maiores contribuidores para alergia e asma, há evidências que o nível aumentado de IL-4 associado à diminuição do interferon gama (IFN-gama) está presente nos asmáticos, IL-5 responsável pela liberação e maturação dos eosinófilos, IL-10 apresenta-se diminuída em pacientes com asma em comparação com controle e é responsável por múltiplas funções dentro da resposta imune e inflamação, assim como a IL-13 encontra-se presente no processo inflamatório e remodelamento de vias aéreas em asmáticos alérgicos (Kinniping et. al., 2017).

A prevalência de asma em crianças é alta, assim como altos índices de absenteísmo são decorrentes das exacerbações e do difícil controle da doença.

Muitas vezes o diagnóstico da asma é inadequado o que leva a um tratamento ineficaz colaborando para piora progressiva da doença. A oscilometria de impulso é uma técnica relativamente nova, apresenta-se como uma medida fidedigna na avaliação de obstrução das vias aéreas tanto proximal quanto distal, seu uso vem crescendo mundialmente e ainda necessita de maiores pesquisas para introduzi-la como auxiliar no diagnóstico de crianças asmáticas.

2. ARTIGO

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IMPULSE OSCILLOMETRY, REMODELING AND INFLAMMATORY AND FIBROTIC MEDIATORS IN ASTHMATIC CHILDREN

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Summary at a Glance: The present study shows for the first time that asthmatic children present increased levels of pro-inflammatory and pro-fibrotic mediators in the breath condensate and in the serum, which correlates with increased airway resistance.

Abstract

Background and objectives: Asthmatic children present variable degrees of airway inflammation, remodeling and resistance, which correlates with disease control and severity. Chronic inflammatory process of the airways trigger airway remodeling, which reflects the degree of airway resistance. Pro-inflammatory and pro-fibrotic mediators are centrally involved in this process. Thus, the present study has investigated for the first time, whether the levels of pulmonary and systemic pro-inflammatory and pro-fibrotic mediators present correlation with the resistance of respiratory system and of proximal and distal airways. **Methods**: 24 asthmatic children (persistent mild and moderate) and 24 non-asthmatic children (both between 6-13 years old) were evaluated for anthropometric characteristics, lung function and mechanics, pulmonary and systemic immune response. **Results**: The results demonstrated that asthmatic children presented increased number of blood eosinophils ($p<0.04$), basophils ($p<0.04$), monocytes ($p<0.002$) and lymphocytes ($p<0.03$). In addition, impaired lung function, as demonstrated by FEV1 ($p<0.0005$) and FEV1/FVC ($p<0.004$), as well as decrease in total resistance of respiratory system (R5Hz; $p<0.009$), increase in the resistance of proximal airways (R20Hz; $p<0.02$), increases in the elastance (Z5Hz; $p<0.02$) and increases in the reactance (X5Hz; $p<0.002$). In addition, the levels of GM-CSF in the breath condensate (BC) ($p<0.0001$) and in the serum ($p<0.0001$); of TGF-beta in the BC ($p<0.0001$) and in the serum ($p<0.004$); IL-4 in the serum ($p<0.0002$) and IL-5 in the BC ($p<0.02$) and in the serum ($p<0.01$) were significantly higher in asthmatic group. **Conclusions**: Impulse oscillometry is a very sensible method to detect airway resistance in asthmatic children, which reflect the airway remodeling, an event followed by increased levels of pro-inflammatory and pro-fibrotic mediators.

Key words: lung function, lung immunology, asthma, asthmatic children.

Short title: impulse oscillometry and remodeling in asthmatic children.

Introduction

Asthma is a heterogeneous disease and is defined as a chronic airway inflammation, leading to a variable airflow limitation, resulting in respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity (Global Initiative for Asthma - GINA 2017). The disease affects more than 334 million people worldwide and, according to the world health organization, 14% of children have ever suffered from any asthma symptom (Global Initiative for Asthma - GINA 2017).

This high rate of asthma prevalence in children, as well as the rates of uncontrolled asthma and asthma exacerbation leads high rates of school absenteeism (Hsu et al., 2016). In addition, acute exacerbations remain one of the main causes of emergency department visits in childhood asthma (Schulze et al., 2016). In this context, innovative methods that could precisely detect the possibility of exacerbations is highly needed. Thus, impulse oscillometry system (IOS) had demonstrated be useful to detect possible exacerbations, since IOS revealed to be much sensible that traditional lung function test, spirometry (Schulze et al., 2016). In addition, some parameters measured by IOS, such as total airway resistance (R20Hz), proximal airway resistance (R5Hz), distal airway resistance (R5Hz – R20Hz) can reflect, when increased, some degree of airway remodeling, as compared with multidetector computed tomography (MDCT) imaging (Brashier and Salvi 2015; Fuso et al., 2018). In fact, airway remodeling is a central feature of asthma and is directly related to disease control and progression (Brashier and Salvi 2015).

Several inflammatory and fibrotic mediators are expressed in the lungs and also in the systemic circulation, such as Th2 cytokines, eicosanoids and

growth factors (Muñoz et. al., 2015). In this context, nitric oxide (NO) is a noninvasive biomarker most widely used in the assessment of airway inflammation, in the respiratory tract it is released by different type of cells (epithelial cells, airway nervous, inflammatory cells and from vascular endothelium), a high concentration of NO is observed in asthmatics when compared with normal individuals. (Muñoz et. al., 2015). In addition, high levels of NO have been associated to lose of control in asthma, hyperresponsiveness and airway remodeling (Matsunaga et al., 2016). In addition, high levels of NO are believed to trigger the release of pro-inflammatory mediators and growth factors, being related to more severe forms of asthma, such as steroid resistant asthma (Eller et al., 2018). It is well known that airway remodeling is mediated by both pro-inflammatory and pro-fibrotic mediators (Ojiaku et al., 2017).

Therefore, the present study investigated for the first time if asthmatic children present impaired lung mechanics related with increased accumulation of pro-inflammatory and pro-fibrotic growth factors.

Material and Methods

Study design

All proceedings performed in this study have been approved by local ethical committee from *Universidade Brasil* (registration number 2.939.688).

Twenty-four asthmatic children and twenty-four non-asthmatic children were recruited and took part in the study, after parents' agreement and signature of the term of consent. To be include into the study, children must had at least ≥ 6 months in clinical treatment and be clinically stable, that is, at least 2

months without exacerbation. The exclusion criteria were to be non-obese [(BMI ≤20) evaluated by BMI and multi-frequential octopolar bioimpedance Bioscan 920 II-S (% Body Fat ≤ 20%) Maltron Inc, Essex, England], non-hypertense, non-dyslipidemic, without cardiovascular diseases, never smokers (even not passive smokers), previous respiratory diseases (except asthma and bronchitis) and neurological diseases. The anthropometric characteristics of children enrolled in the study is presented in Table 1.

Lung Function and Mechanics

Spirometry and impulse oscillometry were performed to measure pulmonary function by using Jaeger Masterscreen pulmonary function instrument (Masterscreen IOS, Erich Jaeger, Hoechberg, Germany) in strict accordance with the American Thoracic Society/European Society of Respiratory Diseases guidelines (ATS/ERS, 2012). The reference values for spirometry were specific for the Brazilian population. Total respiratory impedance (Z5Hz), total resistance of respiratory system (R5Hz), resistance of proximal airways (R20Hz), resistance of distal airways (R5-R20Hz), and pulmonary reactance (X5Hz), resonance frequency (Fres), and respiratory impedance (Z5Hz) were recorded by impulse oscillometry as described previously (Wei et al., 2017).

Exhaled Nitric Oxide

The levels of nitric oxide in the exhaled air was measured by chemiluminescence by using the NOBreath monitor (Bedfont Scientific) (Inoue et al., 2018). The results were expressed in parts per billion (ppb).

Systemic and Pulmonary Immune Response

Five milliliters of venous blood were collected by using vacuum tubes containing EDTA K2 as anticoagulant. The whole blood analysis (white and red series) was performed using the automated system Sysmex 800i (Sysmex Europe GmbH, Germany). Immediately after whole blood analysis, the blood tubes were centrifuged at 1000g, for 7 minutes at 4°C. The serum was stored until the measurements of pro-inflammatory and pro-fibrotic mediators.

For analysis of pulmonary immune response, the levels of pro-inflammatory and pro-fibrotic mediators were measured in breath condensate, which has been collected using RT Tube (Respiratory Research, TX, USA) according to the manufacturer's recommendations.

Thus, the levels of GM-CSF, TSLP, IL-4, IL-5 and TGF-beta was measured both in the serum as well as in the breath condensate, by DuoSet ELISA kits (R&D Systems; MN, USA) through a microplate reader Spectramax I3 (Molecular Devices, CA, USA).

Statistical Analysis

The graphs were built, and the data were analyzed by using SigmaStat 5.0 software (California, USA). Normality of the data was evaluated by the Kolmogorov-Smirnov test. The data were submitted to an unpaired t test for a comparison between the groups. Significance values were adjusted to 5% ($p<0.05$).

Results

Volunteers Anthropometric Characteristics

Table 1 shows the anthropometric characteristics of non-asthmatic and asthmatic children. No significant differences between age, stature, body weight, body mass index (BMI), body composition (% fat, % lean mass, % body water) were found comparing non-asthmatic with asthmatic children ($p>0.05$).

Lung Function

Figure 1 shows the lung function parameters analyzed (Figure 1A and 1B, FVC; Figure 1C and 1D, FEV1; Figure 1E and F; FEV1/FVC; respectively absolute and % of predicted values) comparing non-asthmatic with asthmatic children. The results demonstrated that asthmatic children presented impaired FEV1 ($p<0.0005$) and FEV1/FVC ($p<0.004$) when compared with non-asthmatic children.

Lung Mechanics

Figure 2 shows the lung mechanics parameters analyzed (Z5Hz, X5Hz, R5Hz, R20Hz, R5Hz-R20Hz) comparing non-asthmatic with asthmatic children. The results demonstrated that asthmatic children present impaired impedance of respiratory systems (Z5Hz; $p<0.02$), respiratory reactance (X5Hz; $p<0.002$); proximal airways resistance (R20Hz; $p<0.02$), total resistance of respiratory system (R5Hz; $p<0.009$) and resistance of peripheral/distal airways (R5Hz-R20Hz; $p<0.01$).

Cellular and Humoral Systemic Immune Response

Figure 3 shows the cellular systemic immune response between non-asthmatic and asthmatic children, which has been evaluated by the whole blood analysis. The results demonstrated that asthmatic children presented increased density of lymphocytes (Figure 3D; $p<0.02$ and 3E; $p<0.003$), monocytes

(Figure 3F; $p<0.002$ and 3G; $p<0.001$), eosinophils (Figure 3H; $p<0.008$ and 3I; $p<0.04$) and basophils (Figure 3J; $p<0.04$ and 3K; $p<0.01$).

Figure 4 shows the humoral systemic immune response between non-asthmatic and asthmatic children, which has been evaluated by humoral mediators' measurement in the blood serum. The results demonstrated that asthmatic children presented increased levels of GM-CSF (Figure 4A; $p<0.0001$), TGF-beta (4B; $p<0.004$), IL-4 (Figure 4C; $p<0.0002$), IL-5 (Figure 4D; $p<0.01$) compared with non-asthmatic children, while for TSLP no differences were found ($p>0.05$).

Pulmonary Immune Response

Figure 5 shows the humoral pulmonary immune response between non-asthmatic and asthmatic children, which has been evaluated in the breath condensate (BC). The results demonstrated that asthmatic children present increased levels of pulmonary GM-CSF (Figure 5A; $p<0.0001$); TGF-beta (Figure 5B; $p<0.0001$) and IL-5 (Figure 5D; $p<0.02$), while no differences were found for IL-4 (Figure 5C; $p>0.05$) and TSLP (Figure 5E; $p>0.05$).

Pulmonary Levels of Nitric Oxide

Figure 5F shows that asthmatic children presented increased levels of exhaled nitric oxide compared with non-asthmatic children (Figure 5F; $p<0.02$). The results were expressed in parts per billion (ppb).

Discussion

This study shows for the first time that asthmatic children presenting increased airway resistance, concomitantly present increased levels of pro-inflammatory and pro-fibrotic factors, beyond increased levels of exhaled nitric oxide.

Systemic eosinophilc inflammation has been considered an important cellular biomarker to classify different asthma phenotypes and to predict response to treatments (Sonntag et al., 2019). In summary, blood eosinophils above 300 cells/µl is an important cellular biomarker to predict severe asthma and/or steroid resistant asthma (Sonntag et al., 2019). In the present study, it was observed that the group of asthmatic children enrolled in the study presented values ranging 200 cells/µl until nearly 500 cells/µl, corresponding to values above 6% of eosinophils. In this context, it has been established that such profile of patients presents more severe hyperresponsiveness, bronchospasm and airflow limitation (Castro-Rodriguez et al., 2018). However, in the present study, while we only measure the hyperresponsiveness indirectly using pre and post bronchodilator for spirometry, without a confirmation of hyperresponsiveness, the susceptibility to bronchospasm and airflow limitation were additionally accessed by pre and post bronchodilator response to impulse oscillometry, which has been done for the first time. In this context, the present study found that asthmatic children presented, not only impaired FEV1 and FEV1/FVC response, as classically found, but also impaired lung mechanics, as denoted by impaired impedance of respiratory systems (Z5Hz), respiratory reactance (X5Hz); proximal airways resistance (R20Hz), total resistance of respiratory system (R5Hz) and resistance of peripheral/distal airways (R5Hz-R20Hz). In addition, a slight but novel mechanistic investigation was performed, as demonstrated by increased levels of systemic and pulmonary pro-

inflammatory humoral mediators (GM-CSF, IL-4, IL-5) and pro-fibrotic mediators (TGF-beta).

Increases in the levels of Th2 pro-inflammatory (GM-CSF, IL-4, IL-5) and pro-fibrotic mediators (TGF-beta) are key players in the process of airway remodeling, stimulating hypertrophy and hyperplasia of airway smooth muscle and of airway epithelial cells, leading to exaggerated mucus and extra-cellular matrix proteins production and accumulation (Fehrenbach et al., 2017). In this view, the present study shows for the first time that increased airway resistance in asthmatic children, which reflect functionally the airway remodeling, is followed by increased levels of Th2 pro-inflammatory mediators as well as the growth factor TGF-beta, not only in the systemic circulation, but also into the lungs, as demonstrated in the breath condensate.

Beyond Th2 mediators and growth factors, another mediator named nitric oxide (NO), has been linked to asthma development, severity and exacerbations (Muñoz et al., 2016; Matsunaga et al., 2016; Eller et al., 2017; Ojiaku et al., 2018). NO is an instable gas, considered a highly reactive nitrogen specie, which present a very short half-life, but that can be measured noninvasively in the exhaled air by chemiluminescence, by using bench (Sierra et al., 2019) or portable (Inoue et al., 2018) NO detectors devices. In fact, increased levels of NO have been associated not only with several aspects of asthma (Muñoz et al., 2016; Matsunaga et al., 2016; Eller et al., 2017; Ojiaku et al., 2018), but also with bronchoconstriction-induced by exercise, which is a highly prevalent characteristic of asthmatic patients (Abbasi et al., 2015; Sierra et al., 2019). In the present study, it was found that asthmatic children presented high levels of exhaled NO, ranging among 25-30 ppb, which clearly

indicates an inflammatory process of the airways. However, although these levels of NO already indicate airway inflammation, it was not enough to predict asthma exacerbation or even hyperresponsiveness (Salviano et al., 2018), as observed in the present study (no response ≥ 10% in the FEV1 after bronchodilator). On the other side, these increased levels of NO were associated to increased levels of systemic and pulmonary Th2 cytokines (GM-CSF, IL-4 and IL-5) and also with growth factor TGF-beta. In fact, pre-clinical studies have classically reported that high levels of NO is involved in exacerbated Th2 airway inflammation, remodeling and hyperresponsiveness, while the blocking of inducible nitric oxide synthase (iNOS), responsible for very high levels of NO synthesis, can prevent or even revert this asthmatic phenotype (Th2 airway inflammation, remodeling and hyperresponsiveness) (Angeli et al., 2008; Starling et al., 2009).

Conclusions

We conclude that impulse oscillometry is a very sensible method to detect airway resistance in asthmatic children, which reflect the airway remodeling, an event followed by increased levels of pro-inflammatory and pro-fibrotic mediators, which has been also observed in the lungs, in the breath condensate, as well as systemically, in the serum, in the present study.

Acknowledgements

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Tables

Table 1 - Baseline characteristics of asthmatics and non-asthmatics individuals.

	Asthmatic (n=24)	Non Asthmatic (n=24)	P value
Age (yr)	10 ± 2,27	8,11 ± 1,79	p <0,04
BMI (Kg/ m2)	19,21±6,38	18,86±4,08	p =0,49
Height (cm)	144,61±13,65	135,94±16, 19	p =0,15
Weight	42,15±21,84	36,46±15,1	p =0,88

Abbreviations: BMI, body mass index.
Data are presented as mean±SD.

Figures

Figure 1 – Spirometric Parameters Between Non-Asthmatic and Asthmatic Children

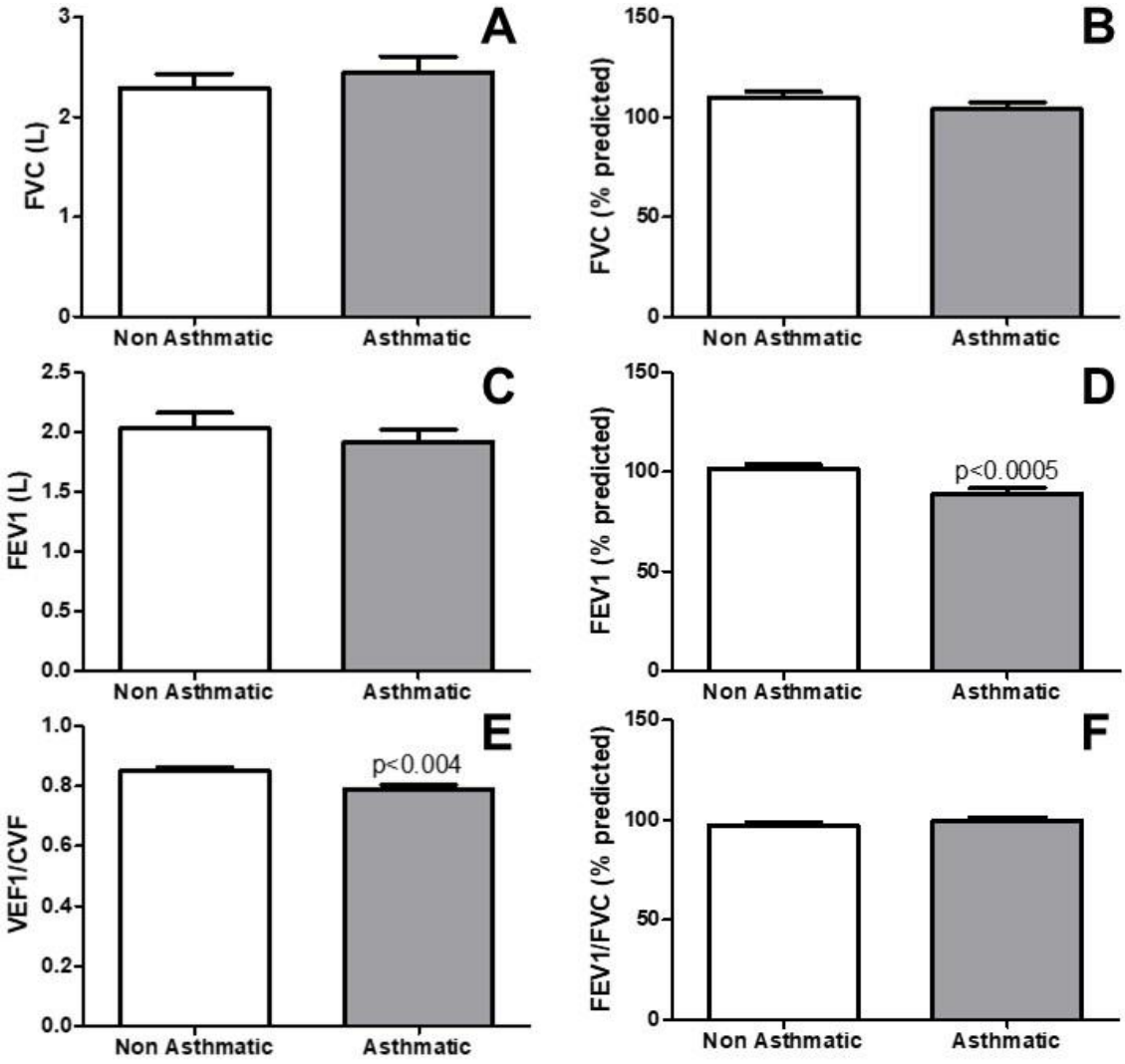

Figure 2 – Oscillometric Parameters Between Non-Asthmatic and Asthmatic Children

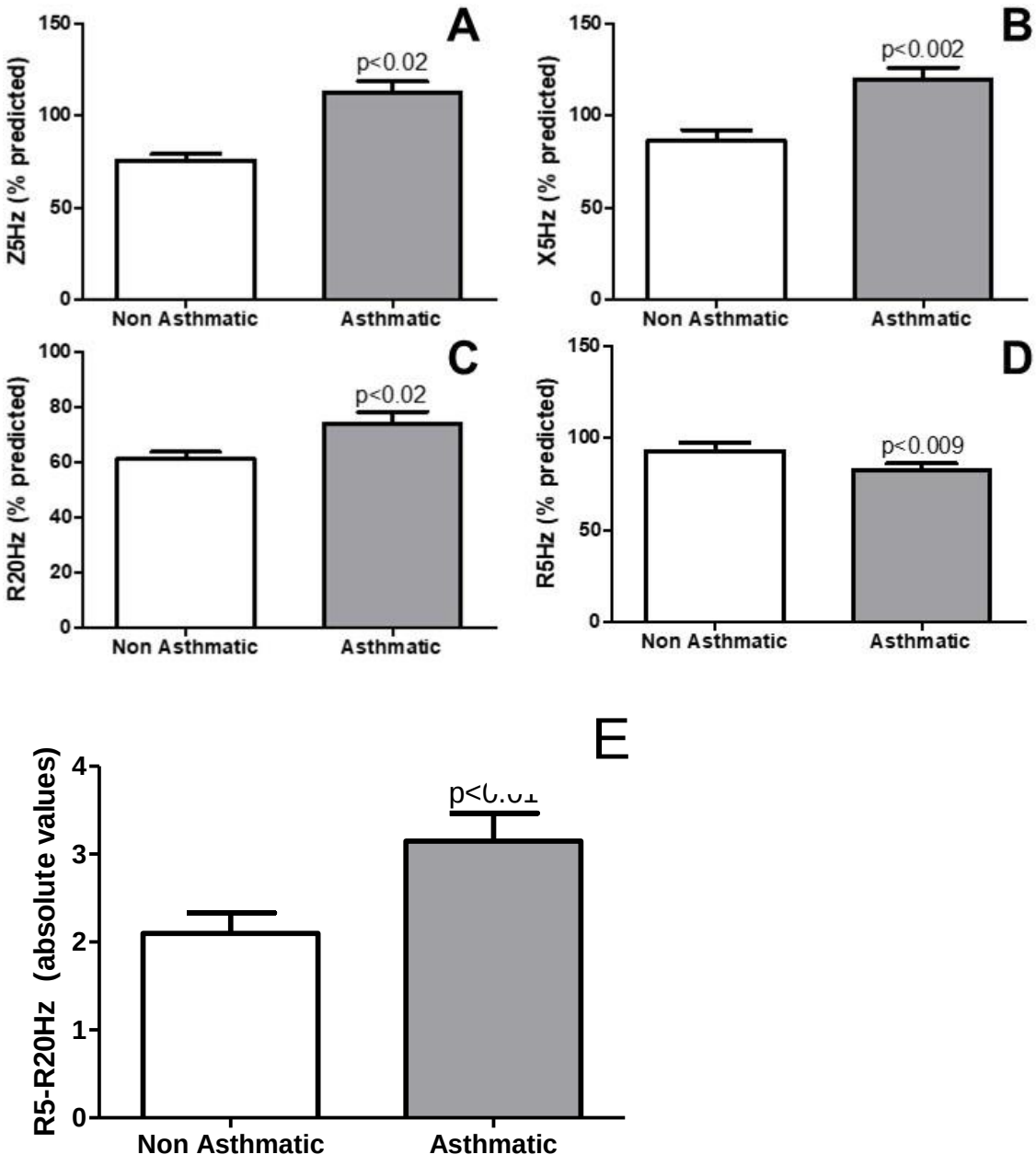

Figure 3 – Whole Blood Analysis of Non-asthmatic and Asthmatic Children

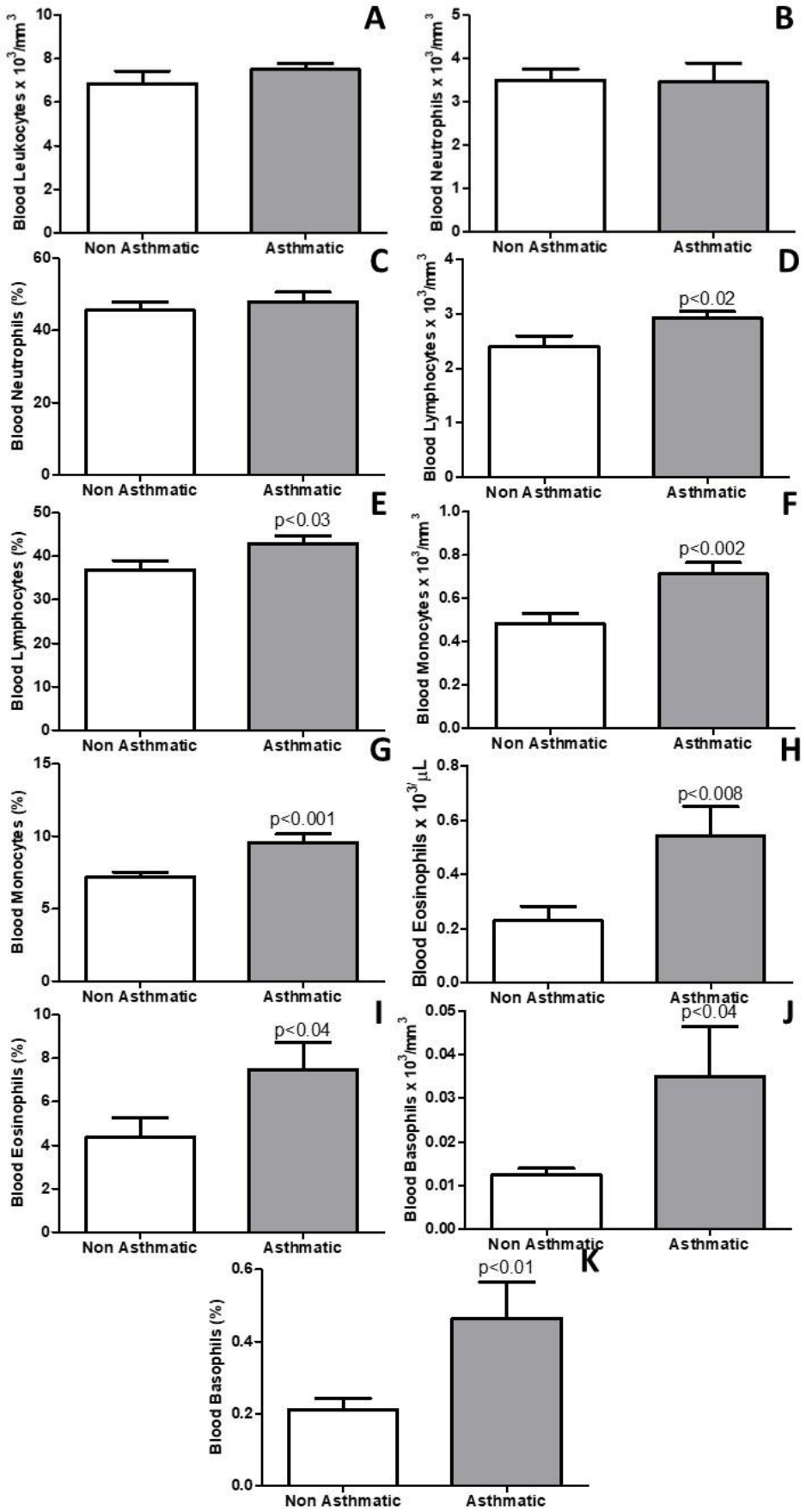

Figure 4 – Analysis of Cytokines and Growth Factor on Serum of Non-asthmatic and asthmatic children

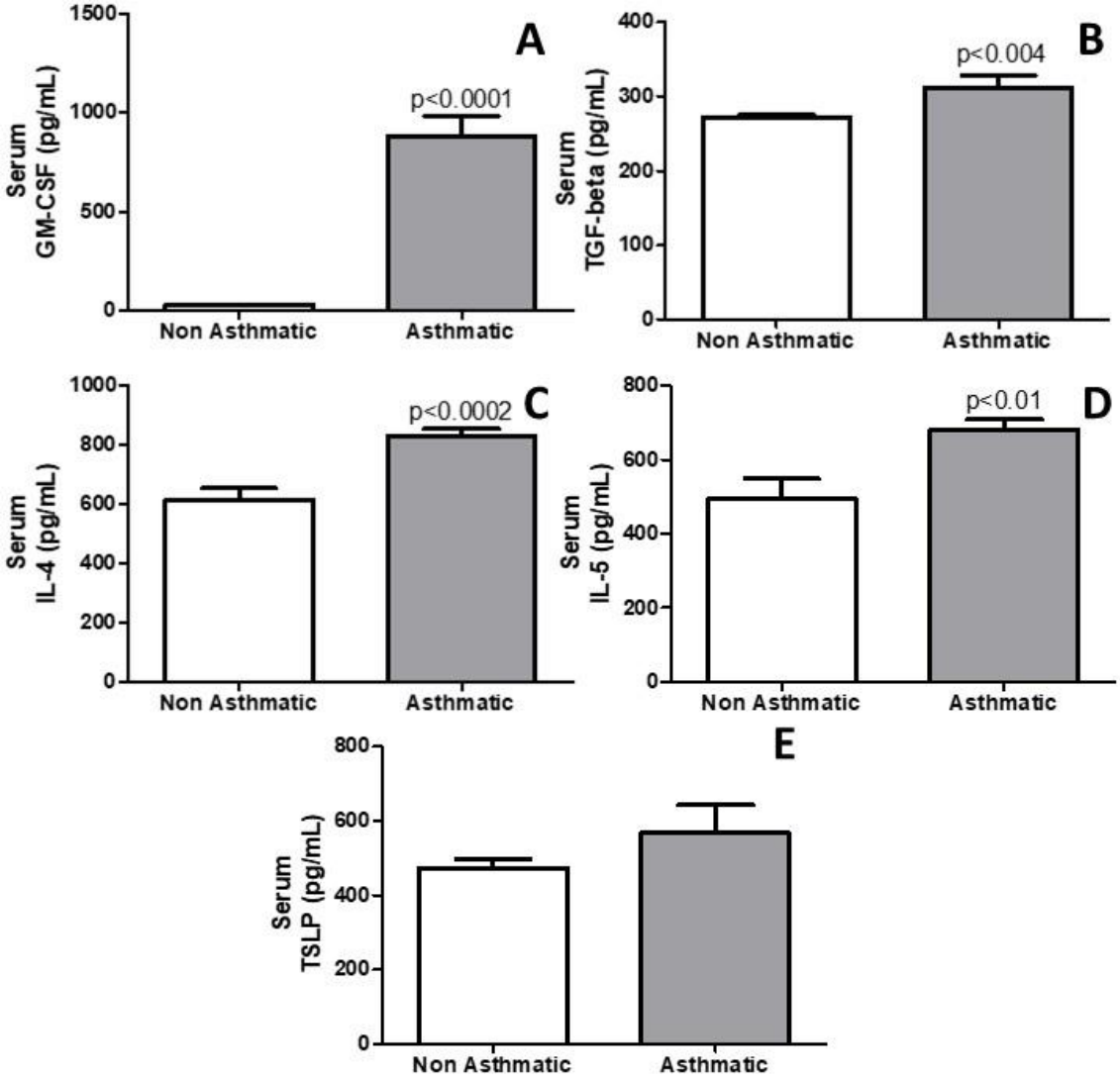

Figure 5 – Analysis of Cytokines and Growth Factor on the Lungs (Breath Condensate) of Non-asthmatic and asthmatic children

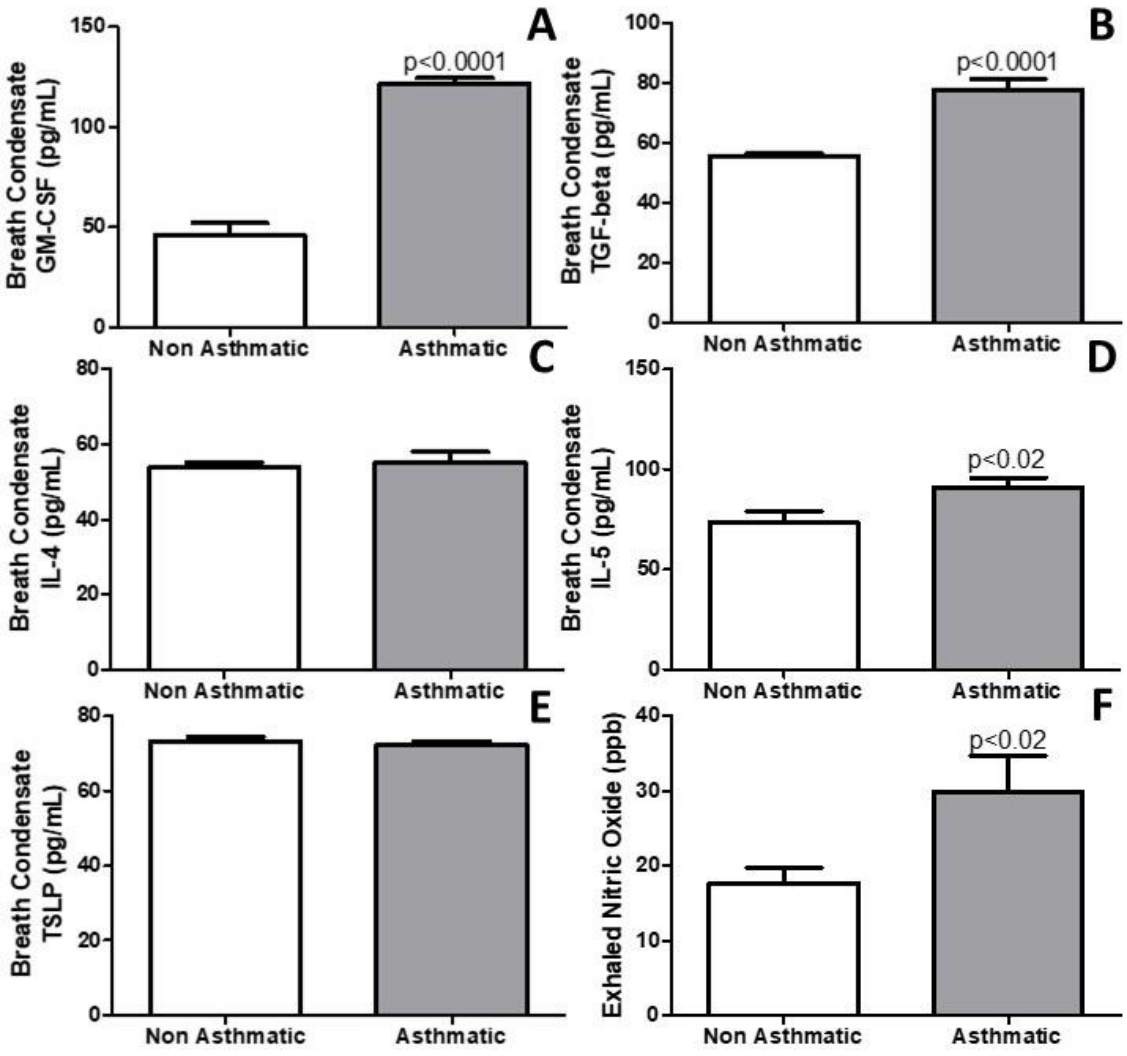

Figure legends

Figure 1. Analysis of lung function by spirometry. Forced vital capacity (FVC); forced expiratory flow in the first second (FEV1).

Figure 2. Analysis of lung mechanics by impulse oscillometry system (IOS). Total respiratory impedance (Z5Hz), total resistance of respiratory system (R5Hz), resistance of proximal airways (R20Hz), resistance of distal airways (R5-R20Hz), and pulmonary reactance (X5Hz), and respiratory impedance (Z5Hz).

Figure 3. Whole blood analysis, including total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils.

Figure 4. Analysis of serum humoral mediators. Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF); transforming growth factor beta (TGF-beta); interleukin 4 (IL-4), interleukin 5 (IL-5), Thymic stromal lymphopoietin (TSLP).

Figure 5. Analysis of pulmonary humoral mediators in the breath condensate. Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF); transforming growth factor beta (TGF-beta); interleukin 4 (IL-4), interleukin 5 (IL-5), Thymic stromal lymphopoietin (TSLP) and exhaled nitric oxide (eNO).

3. CONSIDERAÇÕES FINAIS

A pesquisa iniciou-se em 2018 com a avaliação de 48 crianças, 24 asmáticas provenientes de consultório de pneumologia e 24 crianças saudáveis oriundas da comunidade em São José dos Campos, São Paulo. Foram realizados exames de espirometria, oscilometria, IMC, coleta de sangue e ar condensado.

Foi evidenciado que os indivíduos asmáticos apresentavam redução da função pulmonar quando comparados com as crianças saudáveis. Após a análise da correlação entre os resultados obtidos na oscilometria das crianças asmáticas e os biomarcadores séricos e pulmonares, foi evidenciado que um aumento total da resistência das vias aéreas, as quais mostraram-se mais sensíveis que os parâmetros da espirometria. Além disso, essas alterações observadas na oscilometria foram acompanhadas por elevações dos níveis de fatores pró-inflammatórios e pró-fibróticos, permitindo traçar uma clara correlação entre o aumento da resistência das vias aéreas com o aumento dos níveis séricos e pulmonares de fatores pró-inflamatórios e pró-fibróticos.

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