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## Accuracy of Mid Expiratory Flow and Dysanapsis Parameters for Evaluation of Methacholine Provocation Test

Majid Mirsadraee<sup>1</sup>, Bahareh Forouzesheh<sup>2</sup>, Ehsan Roshandel<sup>2</sup>, Mojtaba Meshkat<sup>2</sup>, Mohammad Hossein Boskabady<sup>3</sup>

<sup>1</sup> Department of Pulmonary Medicine, <sup>2</sup> Zakaria Research Center, Medical School of Mashhad Islamic Azad University, <sup>3</sup> Department of Physiology, Mashhad University of Medical Sciences, MASHHAD-IRAN.

### ABSTRACT

**Background:** The most sensitive parameter for evaluation of airway hyper-responsiveness is PC<sub>35</sub> (35% decrease in specific airway conductance). But assessment of this parameter requires expensive equipments.

This study aimed to evaluate mid expiratory flow and dysanapsis parameters in standard spirometry for possible substitution of PC<sub>35</sub>.

**Materials and Methods:** Thirty-seven subjects with clinical findings suggestive of asthma who had normal standard spirogram were entered in this case-control prospective study. Thirty-seven healthy volunteers were also included in this study as controls.

Airway responsiveness was examined by methacholine challenge test and measurement of PC<sub>20</sub> and PC<sub>35</sub>. In addition, concentration of methacholine needed for 20% reduction in FEF<sub>25-75%</sub>, MEF<sub>50%</sub>, and FEF<sub>25-75%</sub>/FVC was determined and compared with PC<sub>35</sub> as the gold standard. FEF<sub>25-75%</sub>/FVC ratio was used for evaluation of dysanapsis.

**Results:** PC<sub>35</sub> was more sensitive than PC<sub>20</sub> and was obtained in 93% (68/73) of patients in both groups. Although PC<sub>35</sub> and new parameters could be positive in both groups, the concentrations in two groups were significantly different. Regression model showed that in asthmatic patients all the conventional and new parameters had good and comparable correlations with PC<sub>35</sub>. But in the control group alone and in both asthmatic and control groups, PC<sub>20</sub> of FEF<sub>25-75%</sub>/FVC showed a significant correlation with PC<sub>35</sub>. PC<sub>20</sub> of FEF<sub>25-75%</sub>/FVC was also significantly correlated with PC<sub>20</sub> of FEV<sub>1</sub> in asthmatic and control groups. PC<sub>20</sub> of FEF<sub>25-75%</sub>/FVC and MEF<sub>50%</sub> revealed the best accuracy. An equation was determined for calculation of PC<sub>35</sub> according to PC<sub>20</sub> of FEF<sub>25-75%</sub>/FVC and MEF<sub>50%</sub> when PC<sub>35</sub> was unavailable.

**Conclusion:** PC<sub>20</sub> of FEF<sub>25-75%</sub>/FVC ratio and MEF<sub>50%</sub> are sensitive parameters for diagnosis of airway responsiveness. PC<sub>20</sub> of FEF<sub>25-75%</sub>/FVC is the best substitution for PC<sub>35</sub>. (Tanaffos 2009; 8(2): 24-30)

**Key words:** Airway responsiveness, Asthma, Provocation test, Methacholine, Dysanapsis

Correspondence to: Mirsadraee M

Address: No 80, 15th Kosar, Kosar Ave., Vakilabad Blv. Mashhad, Iran.

Postal code : 91786

Email address: mirsadra@mshdiau.ac.ir

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## INTRODUCTION

Diagnosis of asthma requires evaluation of forced expiratory volume in one second (FEV1) and forced vital capacity (FVC). In moderate and severe forms of disease, FEV1 and FEV1/FVC ratio are usually decreased and after bronchodilator therapy FEV1 will improve more than 12% (1). In mild forms of asthma, these parameters are normal and asthma can be proved by detecting the airway hyper-responsiveness usually by performing the methacholine challenge test or exercise test. In measuring airway responsiveness, the usual parameter is the concentration or dose of methacholine causing 20% decrement in FEV1 (PC<sub>20</sub>, PD<sub>20</sub>). Another parameter of airway responsiveness is PC<sub>35</sub> or PD<sub>35</sub>, which is the concentration or dose causing 35% reduction in specific conductance (sGaw). In subjects with hyper-responsiveness, PC<sub>20</sub> or PC<sub>35</sub> are obtained with methacholine concentration less than 8 mg/ml. PC<sub>35</sub> or PD<sub>35</sub> are highly sensitive parameters, but more expensive tests should be performed requiring more complex instruments for measurement (2). In addition, PC<sub>35</sub> can detect large airways' disease whereas PC<sub>20</sub> cannot differentiate between large and small airways (2).

During standard spirometry, mid expiratory flow such as FEF<sub>25-75%</sub> (forced expiratory flow in 25% to 75% of vital capacity that means average flow rate during middle two fourths of the FVC) and MEF<sub>50%</sub> (maximal expiratory flow in 50% of vital capacity) are measured routinely in FVC maneuver and flow volume curve. These tests have a wide normal range; but trend of changes in one patient can show narrow range of variability. These tests evaluate peripheral airways where diseases of chronic airflow obstruction are thought to originate (3). Therefore, these tests could be suitable substitutes for sGaw in measuring airway responsiveness. FEF<sub>25-75%</sub>/FVC

is another useful parameter that can be calculated from routine spirometry. This parameter was used to evaluate airway narrowing in comparison to lung volume, called "*Dysanapsis*" (4,5). FEF<sub>25-75%</sub>/FVC and dysanaptic lung growth have also been evaluated for familial aggregation in families with severe, early onset COPD (6). Accuracy of these parameters was not widely evaluated during methacholine challenge test. It is possible that these parameters can help in better interpretation of methacholine challenge test.

The aim of this study was to evaluate validity of mid flow parameters and dysanapsis parameters in measurement of airway responsiveness, when sGaw is unavailable.

## MATERIALS AND METHODS

### *Patients*

Thirty-seven patients suspected of having asthma and 37 controls were studied for measuring airway responsiveness to methacholine. All of them had normal baseline spirometry and lung volumes. Asthmatic patients mentioned a history of intermittent wheezing or cough exacerbating after exercise or exposure to air pollution or cold air. Night symptoms could aid to diagnosis. A questionnaire regarding the respiratory symptoms was designed and completed. Control subjects were selected from the staff of Ghaem Medical Center and showed PC<sub>20</sub> FEV1 more than 8 mg/ml. They had no past or present history of respiratory complaints or any clinical symptoms or signs of pulmonary disorders. None of the cases or controls had any history of smoking, abnormal pulmonary function test, occupational exposure to air pollution or recent lung infections and they were all cooperative during spirometry.

The methacholine challenge test was performed in both control and asthmatic groups. The Ethical

Committee of the university approved the experiment and each subject gave an informed consent.

**Methods:**

This was a prospective, case-control study performed in pulmonary function laboratory of Ghaem Hospital.

**Techniques and Protocols:**

Subjects were refrained from any drugs and caffeinated drinks for 2 hours before the challenge.

For methacholine challenges, cumulative concentration-response technique was used as previously recommended (7). Methacholine phosphate (molecular weight=196), dissolved in 0.9% sodium chloride solution, was delivered as aerosol from a Wright nebulizer with an airflow of 8 l/min, 2 min for each concentration.

The volume of solution delivered for each concentration was 0.2 ml. The aerosol had a mass median aerodynamic diameter (MMAD) of 3.0  $\mu\text{m}$  as determined by laser light scattering (Malvern Instruments 2600 HSD analyzer, Malvern, U.K.). The same nebulizer was used throughout the experiment. At the beginning of each challenge, baseline FVC maneuver and specific conductance (sGaw) were measured using a body plethysmograph, Sensormedics (Model Vmax 6200, California Co. Ltd., USA). Before FVC maneuver and sGaw measurement, the operator demonstrated the required maneuver, and subjects were encouraged and supervised throughout the test performance. Measurements of FEV<sub>1</sub> and sGaw were performed using the acceptability standards outlined by the "American Thoracic Society" (ATS) (7), in a sitting position inside a box and wearing nose clips. Subjects were instructed to breathe normally in the first step, and then different concentrations of drugs (starting from 0.125 mg/ml methacholine up to 16 mg/ml) were administered. The challenge was terminated when a 20% fall in FEV<sub>1</sub> and/or 35% fall in sGaw were recorded.

**Measurements:**

Standard spirometry was performed in the beginning of the test at least for three times in a sitting position inside the body plethysmograph. FEF<sub>25-75%</sub>/FVC ratio (which is used for describing the dysanapsis of the lung) was calculated.

Methacholine cumulative logarithmic concentration-response curves were constructed for all asthmatic and control subjects and 35% decrement in sGaw (PC<sub>35</sub>) was the gold standard for determining the airway responsiveness. Subjects who showed 20% decrease in FEV<sub>1</sub> (PC<sub>20</sub>) in methacholine concentration less than 8 mg/ml were considered asthmatic while subjects with PC<sub>20</sub> more than 8 mg/ml were entered the control group. New parameters, especially calculated for this study were as follows: methacholine concentration required for 20% decrease in FEF<sub>25-75</sub> (PC<sub>20</sub> of FEF<sub>25-75</sub>), 20% decrease in MEF<sub>50%</sub> (PC<sub>20</sub> of MEF<sub>50%</sub>) and 20% decrease in FEF<sub>25-75</sub>/FVC ratio (PC<sub>20</sub> of FEF<sub>25-75</sub>/FVC) (8).

**Statistics:**

Considering the prevalence of asthma in our region, with alpha risk of 0.05 and potency of 80%, 37 subjects were selected.

Normal distribution of data was checked using Kolmogorov Smirnov test. For comparing quantitative values, unpaired t- test was used. Correlations of new parameters (PC<sub>20</sub> of MEF<sub>50%</sub>, FEF<sub>27-75%</sub> and FEF<sub>25-75%</sub>/FVC ratio) with PC<sub>20</sub> FEV<sub>1</sub> and PC<sub>35</sub> were tested using least square regression. PC<sub>35</sub> was used as the gold standard for measuring the sensitivity, specificity, negative predicted value and positive predicted value of new parameters. EPI INFO 2003 and SPSS 14 software were used for statistical analysis. Significance was accepted at P<0.05.

**RESULTS**

**Baseline values:**

The mean age of asthmatic patients was

37.9±16.8 years with no significant difference when compared to the control group (32.4±16.5 years) (T=0.270, P=0.788). Cough (34/37), wheezing (20/37) and dyspnea (19/37) were the most prevalent symptoms in the asthmatic group. Comparison of baseline FEF<sub>25-75%</sub>/FVC ratio between asthmatic and control groups showed no significant difference (0.82±0.23 and 0.88±0.24, respectively).

#### **Differences between asthmatic and control subjects in new parameters during methacholine challenge test**

The mean ± SD of PC<sub>20</sub> FEV<sub>1</sub> was 8.8±2.3 and 2.46±3.2 mg/ml in control and asthmatic groups, respectively (Table 1). PC<sub>35</sub> was more sensitive than PC<sub>20</sub> and was obtained in 93% (68/73) of both groups. PC<sub>20</sub> FEF<sub>25-75</sub>, FEF<sub>25-75%</sub>/FVC ratio and MEF<sub>50%</sub> less than 8 mg/ml were obtained in 59 (80%), 58 (78%) and 59 (80%) subjects in both groups respectively. Although PC<sub>35</sub> and new parameters could be positive in both groups, the concentrations in two groups were significantly different (Table 1).

Table 1. Comparison of methacholine concentration in asthmatic and control groups in different measured parameters

	Asthmatic	Control	T test	P value
PC <sub>20</sub> FEV <sub>1</sub>	2.46±3.2	8.8±2.3	-9.575	0.0001
PC <sub>20</sub> FEF <sub>25-75</sub>	1.15±2.8	3.86±3.3	-3.704	0.0001
PC <sub>20</sub> MEF <sub>50%</sub>	1.11±2.9	4.34±3.3	-4.335	0.0001
PC <sub>20</sub> FEF <sub>25-75%</sub> /FVC	1.42±3	4.58±4.3	-3.582	0.001
PC <sub>35</sub>	1.16±1.9	3.27±3.2	-3.355	0.001

#### **Correlation between standard and new parameters used for assessing airway responsiveness**

Concentration-response curves of methacholine obtained by measuring specific conductance were closely correlated to those of measuring FEF<sub>25-75</sub>, FEF<sub>25-75%</sub>/FVC ratio, FEV<sub>1</sub> and MEF<sub>50%</sub> (Fig. 1).

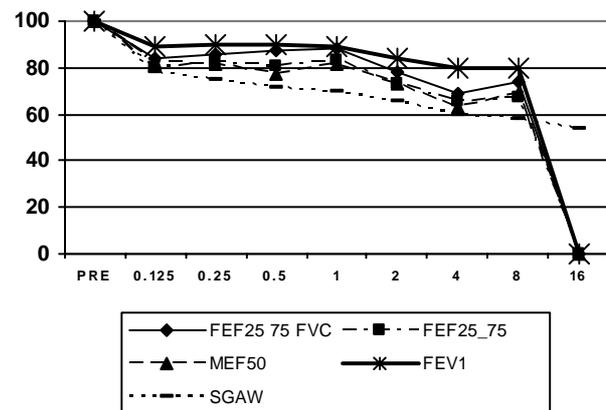


Figure 1. Normalized log concentration-response curves of methacholine by measuring sGaw, FEV<sub>1</sub>, MEF<sub>50</sub>, FEF<sub>25-75</sub> and FEF<sub>25-75</sub>/FVC ratio in asthmatic subjects.

Regression model showed that in asthmatic group all the conventional and new parameters had a good and similar correlation with PC<sub>35</sub>. But in the control group alone and combination of asthmatic and control groups PC<sub>20</sub> of FEF<sub>25-75%</sub>/FVC showed a significant correlation with PC<sub>35</sub> (Table 2). There were significant correlations between PC<sub>20</sub> of FEV<sub>1</sub> and PC<sub>20</sub> of FEF<sub>25-75</sub>/FVC, FEF<sub>25-75</sub> and MEF<sub>50%</sub> in asthmatic patients (r= 0.846, r= 0.827 and r=0.823 respectively; p= 0.0001). In the control group, the only significant correlation was between PC<sub>20</sub> of FEV<sub>1</sub> and PC<sub>20</sub> of FEF<sub>25-75</sub>/FVC (r= 0.454, p=0.005).

Table 2. Correlation of PC<sub>35</sub> and PC<sub>20</sub> of FEF<sub>25-75</sub>, FEF<sub>25-75%</sub>/FVC ratio, FEV<sub>1</sub> and MEF<sub>50%</sub> in subjects evaluated with methacholine challenge test

Correlation of parameters	Asthmatic	Control	Combination
PC <sub>35</sub> and PC <sub>20</sub>	R=0.785	R=0.174	R=0.521
FEV <sub>1</sub>	P=0.0001	P=0.303	P=0.0001
PC <sub>35</sub> and PC <sub>20</sub>	R=0.765	R=0.225	R=0.478
FEF <sub>25-75</sub>	P=0.0001	P=0.18	P=0.0001
PC <sub>35</sub> and PC <sub>20</sub>	R=0.74	R=0.374	R=0.566
MEF <sub>50%</sub>	P=0.0001	P=0.023	P=0.001
PC <sub>35</sub> and PC <sub>20</sub>	R=0.751	R=0.541	R=0.649
FEF <sub>25-75%</sub> /FVC	P=0.0001	P=0.001	P=0.0001

**Accuracy of various parameters used for assessing airway responsiveness**

Accuracy of the new parameters with respect to PC<sub>35</sub> as the gold standard is shown in Table 4. All of them had better sensitivity than PC<sub>20</sub> FEV<sub>1</sub>. PC<sub>20</sub> MEF<sub>50%</sub> and FEF<sub>25-75</sub> /FVC showed the best sensitivity and negative predicted values (98% and 50% respectively).

Table 3. Accuracy of novel parameters for evaluation of airway hyper-responsiveness according to the gold standard of PC<sub>35</sub>

	PC <sub>20</sub> of FEV <sub>1</sub>	PC <sub>20</sub> of FEF <sub>25-75</sub>	PC <sub>20</sub> of MEF <sub>50%</sub>	PC <sub>20</sub> of FEF <sub>25-75</sub> /FVC
Sensitivity	92.8%	98.5%	98.5%	95.7%
Specificity	95%	50%	95%	95%
PPV	95%	98.5%	95%	95%
NPV	16%	50%	50%	25%

PPV= positive predicted value, NPV= negative predicted value

**Calculation of PC<sub>35</sub> according to PC<sub>20</sub> MEF<sub>50%</sub> and PC<sub>20</sub> FEF<sub>25-75</sub>/FVC**

Based on significant correlations between PC<sub>35</sub> and PC<sub>20</sub> of FEF<sub>25-75</sub>/FVC ratio and MEF<sub>50%</sub> in asthmatic group, square regression of PC<sub>20</sub> of FEF<sub>25-75</sub> /FVC and MEF<sub>50%</sub> was determined (r<sup>2</sup>=0.564 and 0.548 respectively). Then an equation was determined for calculation of PC<sub>35</sub> according to PC<sub>20</sub> of FEF<sub>25-75</sub> /FVC and MEF<sub>50%</sub> when PC<sub>35</sub> was unavailable (Equations 1 and 2) (Figure 2).

**Equation 1:**  $PC_{35} = 0.99 + 0.92 \times PC_{20} \text{ of } FEF_{25-75} / FVC$

**Equation 2:**  $PC_{35} = 1.2 + 0.69 \times PC_{20} \text{ of } MEF_{50}$

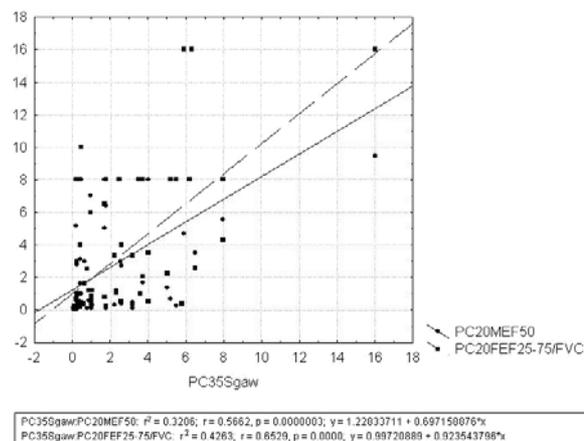


Figure 2. Regression model between PC<sub>35</sub> and PC<sub>20</sub> FEF<sub>25-75</sub>/FVC (◆) and MEF<sub>50%</sub> (■)

**DISCUSSION**

PC<sub>20</sub> FEV<sub>1</sub> and PC<sub>35</sub> are used for evaluation of airway hyper-responsiveness in asthmatic patients. Although specific conductance and other measures were adopted in some early works (9), forced expiratory volume in one second (FEV<sub>1</sub>) is now almost always used for measurement of airway hyper-responsiveness (AHR), because of its greater reproducibility (10,11) and better discrimination between asthmatic and normal subjects (12). Inevitably, whatever maximum dose of histamine or methacholine is permitted and whether or not extrapolation is used, most studies found that less than 50% of the population achieved a 20% fall in FEV<sub>1</sub> (13) (92% in the present study). Specific conductance and airway resistance are more sensitive but require more expensive instruments, and unfamiliarity with the methods used may account for the infrequent use of this test (4). The results of the present study showed that assessing airway responsiveness by measuring FEF<sub>25-75</sub>/FVC ratio and MEF<sub>50%</sub> yields results comparable to PC<sub>35</sub>. In addition, PC<sub>20</sub> FEF<sub>25-75</sub>, FEF<sub>25-75</sub>/FVC ratio, and MEF<sub>50%</sub> could be obtained in more asthmatic subjects than PC<sub>20</sub> FEV<sub>1</sub>. Evaluation of sensitivity,

specificity, positive and negative predictive values also showed that accuracy of new parameters in measurement of airway responsiveness was equal or even better than  $PC_{20}$   $FEV_1$ .

$FEF_{25-75\%}$  and  $MEF_{50\%}$  can measure the flow in the most effort-independent part of the flow volume curve. This portion is very sensitive in peripheral airways, when chronic airflow obstruction is present (14). These two parameters are measured routinely during FVC maneuver and can be used as a sensitive determination of airways reactivity.  $FEF_{25-75\%}$  has a wide normal range in general population, but trend of changes in one patient during measurement of airway reactivity may have low range of variability. Peak expiratory flow rate (PEF) is an effort-dependent test (15).

Goldstein et al. (8) reported that PEF variability was a poor substitute for measurement of airway responsiveness. For this reason, in this study mid expiratory flow ( $FEF_{25-75\%}$  and  $MEF_{50\%}$ ) was used instead of PEF and PEF variability.

Cirillo et al. found a possible role of  $FEF_{25-75\%}$  in predicting airway hyper-responsiveness in airway disorders (16).

Dysanapsis of lung (low size bronchi relative to lung volume) is speculated to be a risk factor for developing childhood wheezing (17). Kanner et al. showed that airway diameter is the best determinant of airway hyper- responsiveness (18); therefore, a parameter such as  $FEF_{25-75\%}/FVC$  has the potential of evaluating AHR better than traditional parameters.

Parker et al. (19) showed that when subjects were classified into four groups according to their  $FEF_{25-75\%}/FVC$  ratio, subjects with lowest ratio also had the lowest  $PD_{20}$ . This finding supports the notion that subjects who are more sensitive to methacholine have smaller airway sizes in relation to their lung size. They concluded that baseline  $FEF_{25-75\%}/FVC$  ratio is a determinant of AHR to methacholine. Borrill et al. (20) suggested that by treating COPD

patients, changes in  $FEF_{25-75\%}$  should be corrected due to the changes in FVC because when FVC improves,  $FEF_{25-75\%}$  shifts along the flow volume loop and may even decline. The concentration causing a 20% fall in  $FEV_1$ , showed greater reproducibility and better discrimination between asthmatic and normal subjects (21). In our study we used a similar schedule (20% reduction for all parameters) for assessing the change in new parameters, but another study is required for determining the likelihood ratio.  $PC_{20}$   $FEF_{25-75\%}/FVC$  can easily be measured by routine spirometry. We showed that  $FEF_{25-75\%}/FVC$  ratio and  $PC_{20}$   $MEF_{50\%}$  are sensitive parameters for diagnosis of AHR. Therefore,  $PC_{20}$   $FEF_{25-75\%}/FVC$  and  $MEF_{50\%}$  are good substitutes for  $PC_{35}$  and can be calculated according to equations mentioned above.

In conclusion, the results of the present study showed that measurement of  $PC_{20}$   $FEF_{25-75\%}/FVC$  ratio and  $MEF_{50\%}$  are much easier than  $PC_{35}$  and need cheaper equipments which are available in most general hospitals. These parameters have equivalent values to  $PC_{35}$  in measurement of airway responsiveness in asthmatic patients.

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