TANAFFOS

Determination of a Cut-off Point for Exhaled Nitric Oxide in the Diagnosis of Asthma in an Iranian Population

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Background: Asthma is a major source of global social and economic burden; thus, its early detection is important. Measurement of fractional exhaled nitric oxide (FENO) has been used recently considered a good indicator of asthma and also a sensitive and non-invasive method for monitoring airway inflammation. This study was conducted to determine the cut-off point of FENO for the diagnosis of asthma in the studied population.

Materials and Methods: The subjects of this cross-sectional diagnostic study were assessed by the FENO test, spirometry, and methacholine challenge test. The best cut-off point of the FENO for the diagnosis of asthma was determined. The data were analyzed by SPSS 20 using student t-test, and Chi-square test and the ROC curves were also drawn.

Results: The mean FENO in asthmatic and non-asthmatic subjects was 43.5±33.41 and 17.5±21.48 ppb, respectively (P <0.001). The best cut-off point of the FENO based on the overall sensitivity and specificity was 39.5 ppb.

Conclusion: According to the results of this study, symptomatic patients with FENO higher than 39.5 ppb could be considered as asthmatic.

Key words: Cut-off Point, Exhaled Nitric Oxide, Asthma

INTRODUCTION

The prevalence of asthma is constantly increasing and it has become a major source of global social and economic burden. Various guidelines have been published for the diagnosis and management of asthma; however, accurate diagnosis of asthma remains difficult in practice for physicians (1, 2). Asthma is usually diagnosed based on the patient's symptoms and the presence of reversible airway obstruction or airway hyper-responsiveness (3).

In many patients with mild asthma, lung function is normal, which makes it difficult to assess the reversibility. The results of the airway hyper-responsiveness test have high sensitivity and diagnostic value, but this test is time-consuming, relatively aggressive, and sometimes causes an

asthma attack (4). Although these methods in patients with asthma are linked to airway inflammation but do not directly indicate airway inflammation. Sampling the airway mucosa and bronchoalveolar lavage by bronchoscopy can identify inflammation in the airways; however, this method is invasive and not suitable as a routine test (5, 6). Measurement of fractional exhaled nitric oxide (FENO) has been used recently considered a good indicator of asthma and also a sensitive and non-invasive method for monitoring airway inflammation (7).

Nitric oxide (NO) is a gas molecule emitted by the human respiratory tract and may indicate the inflammation of the airways (8, 9). Studies have reported that FENO levels in patients with asthma are linked to

airway inflammation, airway hyper-responsiveness, the number of eosinophils in induced sputum, and lung function (7, 10, 11). Current guidelines of the American Thoracic Society (ATS) suggest the use of FENO for the diagnosis of eosinophilic airway inflammation (9).

FENO levels are affected by different factors in a population; therefore, the reference range and cut-off points for clinical applications must be specifically determined separately for each population (12, 13). Various studies have been conducted using FENO to monitor asthma treatment routinely in developed countries.

We could not find a study on the diagnostic value of FENO in the diagnosis of asthma and its cut-off points FENO in Iran. Accordingly, in this study, we aimed at determining the cut-off point of FENO for the diagnosis of asthma in the studied population.

MATERIALS AND METHODS

This cross-sectional diagnostic study was conducted on 18-year-old and older patients with at least one of the following respiratory signs: cough, shortness of breath, and chest tightness. The patients were selected consecutively from a lung clinic of Shahid Sadoughi hospital and occupational medicine clinic of Shahid Rahnamoun hospitals in one year.

Exclusion criteria included inability to perform acceptable FENO or spirometry maneuvers, smoking or being a former smoker during the past year, occupational respiratory exposure, acute respiratory infection six weeks before the study, chronic lung diseases, consumption of oral or inhaled corticosteroids, NO-releasing drugs (e.g., isosorbide dinitrate, trinitroglycerin, sildenafil, etc.), and treatment with effective medications on leukotriene (montelukast and zafirlukast, etc.). A standard questionnaire, spirometry with bronchodilator administration, and methacholine challenge test (MCT) were used to diagnose asthma.

At first, the subjects with respiratory symptoms were evaluated by the Venable questionnaire (14), along with a few additional questions about chronic respiratory symptoms, age, sex, employment duration, history of lung diseases and smoking, the presence of respiratory exposures and personal family history of asthma or atopy. At least, three positive answers in the Venable questionnaire with a sensitivity of 65-91% and specificity of 85-96% could detect asthma or, at least increased responsiveness of the airways (14). The questionnaire was filled out by the patients under the supervision of a physician.

To measure exhaled nitric oxide (FENO), we used the NO breath device (Bedfont, Germany). The maneuvers were performed according to ATS guidelines (15). To measure FENO, the patients were asked to do a deep inspiration to reach the full capacity of the lung (total lung capacity), and then immediately, send out the air through the mouthpiece at a constant speed as much as possible. This was repeated at least three times and the average of the results was recorded.

After recording the values of FENO, spirometry was performed for all patients. Spirometry was performed in a sitting position and using Spirolab III (Mir, Italy), in accordance with the standards of ATS / European Respiratory Society (ERS) (12). To measure lung volumes by the spirometry device, the person was asked to use a nose clip and put the mouthpiece in the mouth, and after a very deep inspiration, performed a rapid expiration with maximum intensity. For each person, at least three acceptable maneuvers met the repeatability standards of ATS/ERS (12).

For patients with obstructive pattern in spirometry, the post-bronchodilator test was performed; i.e. 15 min after administration of 400 µg of inhaled Salbutamol (4 puffs Ventalex, Sina Darou, Tehran, every 30 s), spirometry was performed again in the same condition.

Values of the forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) before and after using a bronchodilator were compared. Patients who responded to bronchodilator according to the ATS guidelines (12% and 200 cc increase in FEV_1 or FVC) were

diagnosed as suffering from asthma. Those who did not respond to bronchodilator therapy were treated with an inhaled corticosteroid, and spirometry was repeated 4-6 weeks later to confirm or reject asthma (15).

For patients who initially had normal spirometry, MCT was performed (device: Provocation test 2, Pari, Germany). The MCT was performed according to the ATS guidelines (12). For this purpose, a baseline spirometry was performed without medication and when saline and various concentrations of methacholine (from the lowest level) were administered to the subject by a nebulizer. The patient used a nose clip, and spirometry was performed 30 and 90 s after administration of different concentrations of methacholine, and FEV_1 was recorded. If a decline in FEV_1 after each concentration was 20% or more, the same level was assumed as diagnostic level and test was stopped. After 10 min of waiting, spirometry was repeated to confirm or reject the diagnosis (15). Then, the subjects were divided into two groups: with asthma and without asthma and FENO levels were compared between the two groups.

The study was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences. Informed consent was obtained from each participant.

The data were analyzed by SPSS software (version 20) using a student t-test and Chi-square test. The ROC curves were also drawn to find the best cut-off points of the FENO.

RESULTS

A total of 87 patients (59.7% male) with a mean age of 34.5±5.7 years (range =18 -77 years) were studied. The most common respiratory symptoms were wheezing (72.4%), cough (66.6%), and dyspnea (63.2%). In addition, 25.2%, 26.4%, and 22.9% of the participants had a history of childhood asthma, allergic rhinitis, and atopy (Table 1).

Also, 49 patients had a positive bronchodilator test, and asthma was confirmed in 21 patients with MCT. Finally, 80.45% had asthma and in 19.55% of the participants, asthma was rejected (Table 1). The mean FENO in asthmatic and non-asthmatic subjects was 43.5±33.41 and 17.5±21.48 ppb, respectively. This difference was statistically significant (P <0.001).

Figure 1 shows the ROC curve for FENO in all subjects. The best cut-off point of FENO based on the overall sensitivity and specificity was 39.5 ppb. The cut-off point of 39.5 has 48% sensitivity and 94% specificity, 97% positive predictive value, and 30% negative predictive value.

Table 1. The frequency (number, percent) of different characteristics of the study population

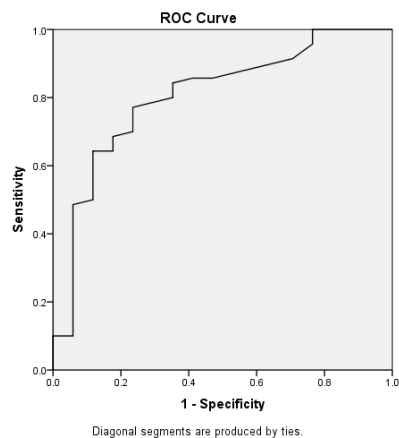|		Asthmatic	Non asthmatic	Total
Sex	Females	30(34.48)	5(5.74)	35(40.20)
	Males	40(45.97)	12(13.79)	52(59.77)
	All subjects	70(80.45)	17(19.55)	87(100)
Signs & symptoms	Dyspnea	54(62.06)	14(16.09)	68(78.16)
	Cough	55(63.21)	12(13.79)	67(77.00)
	Wheezing	60(68.96)	11(12.64)	71(81.60)
	Atopy	18(20.68)	2(2.30)	20(22.98)
	Allergic rhinitis	19(21.83)	4(4.59)	23(26.43)
	Childhood asthma	21(24.13)	1(1.2)	22(25.23)

Figure 1. ROC curve analysis in all subjects

At the cut-off point of 97.5 ppb, all healthy individuals were correctly diagnosed (specificity= 100%), but 63 patients (90%) with asthma were considered healthy (sensitivity = 10%); thus, due to the features of this cut-off point, this level was not suitable for diagnosis. At the cut-off point of 6.5, all patients were correctly diagnosed (sensitivity 100%), but 76% of subjects with FENO above the cut-off point were healthy and did not have asthma (specificity 24%) (Table 2).

Table 2. Sensitivity and specificity of different cut-off points of FENO

FENO (ppb)	Sensitivity (%)	Specificity (%)
4.50	100	11.8
6.50	100	23.5
11.50	85.7	41.2
16.50	77.1	76.5
18.50	75.7	76.5
20.50	70	76.5
29.00	62.9	88.2
36.00	52.9	88.2
37.50	51.4	88.2
38.50	50.0	88.2
39.50	48.6	94.0
40.50	44.3	95.0
41.50	42.9	95.0
42.50	41.4	95.0
48.50	30.0	95.0
50.50	28.6	95.0
65.50	18.6	95.0
83.50	11.4	95.0
97.50	10.0	100
105.00	7.1	100
151.00	00.0	100

To determine the cut-off point by age, patients were divided into two groups according to mean age (34.5 years), a group below the age of 34.5 years (45 cases), and another group (42 cases) older than 34.5 years. Based on the ROC curve analysis, the cut-off point in the former age group was 39.5 ppb and in the latter was 17.5 ppb (Figures 2 and 3). FENO cut-off point was determined 17.5 ppb and 39.5 ppb for females and males, respectively (Figures 4 and 5).
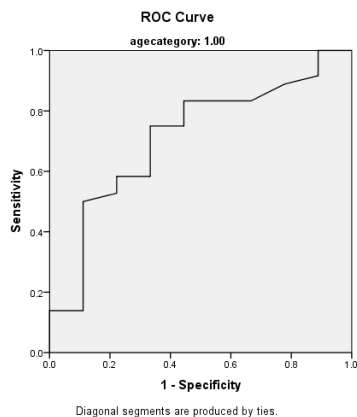
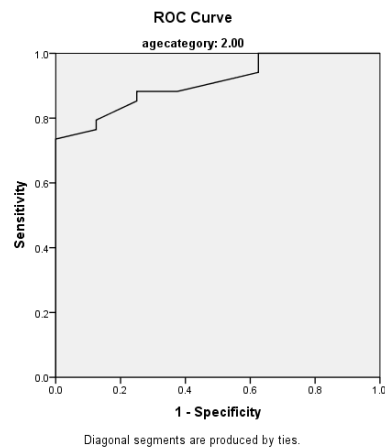**Figure 2.** ROC curve analysis in the age of less than 34.5 years

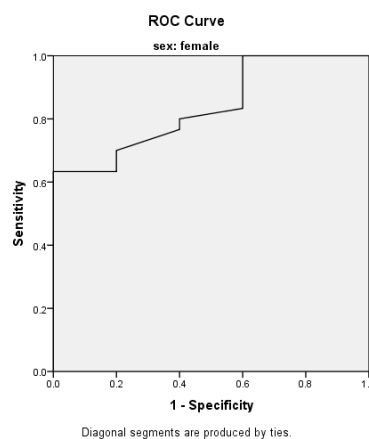**Figure 3.** ROC curve analysis in the age of above 34.5 years

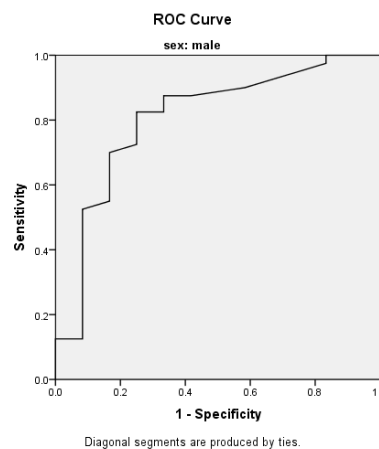**Figure 4.** ROC curve analysis in females.

Figure 5. ROC curve analysis in males.

DISCUSSION

In this study, using a portable FENO analyzer, the FENO cut-off point in an Iranian population in Yazd, a city in the center of Iran, was obtained. Our results showed that

the best cut-off point of FENO for detection of asthma is 39.5 ppb. The cut-off point was 39.5 ppb in males and 17.5 ppb in females. In addition, for cases aged younger than 34.5 years, the cut-off point was 39.5 ppb, but for those over 34.5 years, it was 17.5 ppb.

Also, based on the need for different levels of sensitivity and specificity, some NO levels were determined, which can be used in clinical evaluation. Our results showed that the cut-off points of FENO are significantly different in both genders, which reflects the significant impact of gender.

In a study in 2014, the cut-off point for the diagnosis of asthma was 30.5 ppb for males and 20.5 ppb for females, and gender was shown as an independent determinative factor for the FENO (16). They showed that weight, height, and body mass index (BMI) can affect the levels of exhaled NO, but in our study, BMI, height, and weight were not included. The results of our study are consistent with this study in terms of gender differences, and the differences in cut-off points observed in our study could be due to differences in diet, lifestyle, height, and weight that were not studied.

Zitt showed that exhaled FENO in asthmatic patients was 2-3 times higher than the normal subjects (17). Our results showed a significant difference in NO in asthmatic patients and non-asthmatics.

Pedrosa et al. reached the cut-off point of 40 ppb for NO for asthma diagnosis (18), which is consistent with our results. In another study on Chinese adults 18-90 years old, the average FENO of 32.6 ppb was determined. In this study, FENO levels in men were more than in women. According to this study, not only in asthmatic patients but also in the general population, FENO levels were higher in men than women and different cut-off points were expected in both genders (19).

CONCLUSION

In summary, according to the results of this study, symptomatic patients with high levels of exhaled FENO more than 39.5 ppb could be considered as asthmatic.

Accordingly, it should be noted that both age and gender are effective factors; however, further studies are required to prove the effectiveness of the results. It is essential to interpret FENO levels regarding age and gender.

Acknowledgments

REFERENCES

1. Global Initiative for Asthma Management and Prevention: NHLBI/WHOWorkshop Report. Bethesda, MD: National Institutes Heart, Lung, and Blood Institute; 2010.

2. Asthma Prevention and Management Guideline, Japan 2009. Tokyo, Japan: Japanese Society of Allergology; 2009.

3. Hunter CJ, Brightling CE, Woltmann G, Wardlaw AJ, Pavord ID. A comparison of the validity of different diagnostic tests in adults with asthma. *Chest* 2002;121(4):1051-7.

4. 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(1):309-29.

5. Saito J, Inoue K, Sugawara A, Yoshikawa M, Watanabe K, Ishida T, et al. Exhaled nitric oxide as a marker of airway inflammation for an epidemiologic study in schoolchildren. *J Allergy Clin Immunol* 2004;114(3):512-6.

6. Sato S, Saito J, Sato Y, Ishii T, Xintao W, Tanino Y, et al. Clinical usefulness of fractional exhaled nitric oxide for diagnosing prolonged cough. *Respir Med* 2008;102(10):1452-9.

7. Lim S, Jatakanon A, Meah S, Oates T, Chung KF, Barnes PJ. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. *Thorax* 2000;55(3):184-8.

8. Barnes PJ, Belvisi MG. Nitric oxide and lung disease. *Thorax* 1993;48(10):1034-43.

9. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline:

interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184(5):602-15.

10. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;53(2):91-5.

11. Sandrini A, Taylor DR, Thomas PS, Yates DH. Fractional exhaled nitric oxide in asthma: an update. *Respirology* 2010;15(1):57-70.

12. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171(8):912-30.

13. Sandrini A, Taylor DR, Thomas PS, Yates DH. Fractional exhaled nitric oxide in asthma: an update. *Respirology* 2010;15(1):57-70.

14. Venables KM, Farrer N, Sharp L, Graneek BJ, Newman Taylor AJ. Respiratory symptoms questionnaire for asthma epidemiology: validity and reproducibility. *Thorax* 1993;48(3):214-9.

15. American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;171(4):388-416.

16. Jo EJ, Song WJ, Kim TW, Park HW, Chang YS, Kim TB, et al. Reference ranges and determinant factors for exhaled nitric oxide in a healthy korean elderly population. *Allergy Asthma Immunol Res* 2014;6(6):504-10.

17. Zitt M. Clinical applications of exhaled nitric oxide for the diagnosis and management of asthma: a consensus report. *Clin Ther* 2005;27(8):1238-50.

18. Pedrosa M, Cancelliere N, Barranco P, López-Carrasco V, Quirce S. Usefulness of exhaled nitric oxide for diagnosing asthma. *J Asthma* 2010;47(7):817-21.

19. Ko FW, Leung TF, Wong GW, Chu JH, Sy HY, Hui DS. Determinants of, and reference equation for, exhaled nitric oxide in the Chinese population. *Eur Respir J* 2013;42(3):767-75.