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Exhaled nitric oxide atopy, and spirometry in asthma and rhinitis patients in India

The authors declare no financial disclosure

Abstract

Introduction: Asthma is a chronic airway inflammatory disorder. Nitric oxide (NO) is non-invasively measured in exhaled breath (FeNO). The aim of the study was to investigate the anthropometric and physiologic factors that influence FeNO measurements. Also, to evaluate FeNO correlation with spirometry and inflammatory markers in asthma and rhinitis.

Material and methods: The study was a prospective analysis of asthma (BA) and rhinitis (AR) in patients enrolled from outpatient clinics between 2011 and 2015. Healthy controls (HC) were enrolled from the community. All subjects underwent baseline spirometry with reversibility, FeNO measurements, skin prick tests, and blood sampling for absolute eosinophil counts and serum total IgE levels.

Results: Of 528 enrolled participants, 215 were BA, 248 were BA-AR and 65 were HC. The mean FeNO was higher in atopic versus nonatopic subjects (34.14 vs. 25.99; $p < 0.001$); asthmatics versus non-asthmatics (30.46 vs. 12.91; $p < 0.001$), and in participants with BA-AR, compared to those without BA-AR (32.56 vs. 30.46; $p < 0.001$). The odds ratio for FeNO in the study population showed a significant positive association with male gender, absolute eosinophil count (AEC), breathlessness, duration of symptoms, family history and atopy. In examining the diagnostic accuracy of FeNO for asthma, the AUC for FeNO value is 0.833 (95% confidence interval [CI], 0.717–0.901), with cut-off levels to screen for asthma being 19.45 at 71.2% sensitivity and 81.8% specificity ($p < 0.001$). The Positive Predictive Value 96.84% (95% CI: 94.43–98.23) and Negative Predictive Value 30% (95% CI: 23.78–37.05) for asthma prediction with FeNO.

Conclusion: The study highlights the importance of estimation of anthropometric parameters and dyspnea assessment in the evaluation of FeNO levels. Also, the presence of atopy may influence the results in the interpretation of FeNO readings. Moreover, the study have demonstrated that spirometry and FeNO have no significant correlation, which further lays emphasis on them as being different physiological parameters of asthma.

Key words: asthma, rhinitis, atopy, FeNO

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Introduction

Globally, asthma affects approximately 300 million individuals with prevalence varying from 1% to 18% in different geographical regions [1, 2]. In India, the prevalence in adults varied from 0.96% to 11.03%, while in children ranged from 2.3% to 11.9% [3].

Asthma is a chronic airway inflammatory disorder, and assessment of airway inflammation may have therapeutic implications. The nitric oxide (NO) in the lung/airways has a key role as a vasodilator, bronchodilator, neurotransmitter, and inflammatory mediator [4]. NO is non-invasively measured in exhaled breath (FeNO) and has gained substantial, clinical, and scientific interest for diagnosis, monitoring, or predicting the response to the treatment of airway inflammation in asthma [5]. Allergic rhinitis patients have increased FeNO levels, reflecting the extension of

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inflammation throughout the airways, a feature in the well-known concept of the 'united airway' [6].

The FeNO level is influenced by anthropometric variables such as age, sex, height, weight and body mass index (BMI). Age-dependent increase in FeNO levels has been observed; higher levels have been found to be in men than in women [7–9]. FeNO significantly correlates with height, due to increased lung size with taller height [10]. The relationship between BMI and FeNO in adults has been inconclusive [11].

There is a convincing evidence of an association between atopy and FeNO; a positive correlation between FeNO and the number of positive skin prick tests has been reported [12–14]. Also, serum total IgE (S.T.IgE) correlated with FeNO levels in asthmatics [15]. However, the correlation of FeNO with spirometry variables has not been clearly defined [16, 17].

The objective of this study was to observe anthropometric and physiological factors that influence FeNO measurements along with its relation to spirometry and inflammatory markers in the Indian population, specifically in those with asthma and rhinitis.

Material and methods

This study is a prospective analysis of asthma patients performed over a period of 4 years, between 2011 and 2015.

Study design and demographics

The diagnosis of asthma and allergic rhinitis were based on the Global Initiative for Asthma (GINA), and Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines respectively [18, 19]. The patients diagnosed with bronchial asthma (BA) and bronchial asthma with allergic rhinitis (BA-AR) were enrolled for the study from the outpatient clinics. Healthy controls (HC) were enrolled from the community and enquired of previous episodes of asthma or any physician-based diagnosis of asthma; and only those with negative answer were included in the study.

The subjects with the inability to satisfactorily perform the nitric oxide (NO) maneuver were excluded. Other exclusion criteria were the following: 1) Smoker (former and current smokers); 2) Inhaled/nasal/oral steroid intake in the preceding 1 month; 3) Episode of upper or lower respiratory tract infection in the preceding 1 month; and 4) History of urticaria/eczema.

All subjects underwent investigations, including baseline spirometry with reversibility, FeNO measurements, skin prick tests, and blood sampling for absolute eosinophil counts, and serum total IgE levels.

To participate in the study, written informed consent was obtained from all subjects/parents (in the case of subject's age < 18 years). The institutional ethical committee approved the study protocol.

Measurement of FeNO

The measurement of exhaled NO was performed using NIOX chemiluminescence analyzer (Aerocrine AB, Solna, Sweden) in accordance with the 2005 ATS/ERS recommendations [20].

The patient inserted the mouthpiece, inhaled through the mouth to total lung capacity (TLC) and immediately exhaled at a constant flow rate (50 ml/s) to residual volume without breath-holding. The duration of exhalation had to be sufficient (> 4 seconds in subjects < 12 years and > 6 seconds in subjects > 12 years). Repeated, reproducible exhalations were performed to obtain at least 2 NO plateau values that agreed within 10% of each other. The mean level of two reproducible recordings was used as the result value.

Skin prick testing (SPT)

SPT to 58 common aeroallergens was performed in all the patients as per standard guidelines to assess for atopy [21]. These are the most common aeroallergens in the clinical practice of allergy in India [22]. Atopy was defined as a positive skin prick test (wheal diameter of > 3 mm as compared to buffer saline as a control) for at least ≥ 1 aeroallergen [21].

Measurement of serum total IgE and absolute eosinophil counts

Serum Total IgE was estimated by ELISA method using MINILYSER-TECAN, Austria, Calbiotech kit as per manufacturer's instructions. The number of peripheral blood eosinophils was counted in EDTA-containing blood samples using an automated analyzer.

Spirometry with reversibility

Spirometry was performed on a dry, rolling-seal spirometer of the Benchmark model lung function machine (P.K. Morgan, Kent, UK). Maximal Expiratory Flow Volume curves were obtained as per the ATS recommendations before and after bronchodilation. At least 3 technically acceptable forced expirations were performed for up to 8 tests. The highest forced vital capacity

(FVC) and forced expiratory volume in 1 second (FEV_1) were recorded and percentages of the predicted values were calculated [23]. An improvement in FEV_1 and FVC of at least 12% after bronchodilator, compared with the baseline value, indicates a positive bronchodilator response [23]. The severity of spirometric abnormality was classified on the basis of forced expiratory volume in 1 second (FEV_1) [23].

Statistical analysis

Data analysis was performed using SPSS statistical package version 15.0 for Windows (SPSS, Chicago, IL, USA). It was examined for distribution, and homogeneity of variances was checked before applying parametric tests. Quantitative variables were compared between 3 groups using ANOVA / Kruskal-Wallis test and between 2 groups using unpaired t-test/Mann-Whitney test. Qualitative variables were compared using Chi-square/Fisher's exact test. Statistical significance was set at the conventional 5% level ($p < 0.05$). The univariate analysis of factors associated with FeNO was done using Pearson correlation. The conventional 5% level ($p < 0.05$) was considered to be statistically significant. To assess the diagnostic accuracy of FeNO to predict atopy, we constructed receiver-operating-characteristic (ROC) curves and calculated the areas-under-the-curve (AUC) using 5-fold cross-validation.

Results

Characteristics of the study population

Of 528 enrolled participants, 215 were BA, 248 were BA-AR and 65 were healthy controls. The clinical characteristics of the study population have been described in Table 1. The spirometry parameters of the study population have been described in Table 2.

Factors associated with FeNO

Men had higher FeNO values (34.14 vs. 26.16; $p < 0.001$) than women. The mean FeNO was higher in atopic than in non-atopic patients (34.14 vs. 25.99; $p < 0.001$), higher in asthmatics than in non-asthmatics (30.46 vs. 12.91; $p < 0.001$) and higher in participants with allergic rhinitis compared with those without this disorder (32.56 vs. 30.46; $p < 0.001$). The odds ratio for FeNO in the study population showed a significant positive association with male gender, absolute eosinophil count (AEC), breathlessness, duration of symptoms, family history and atopy (Table 2).

Diagnostic accuracy of FeNO for asthma and atopy

In determining the diagnostic accuracy of FeNO for asthma, the AUC for FeNO value was 0.833 (95% CI, 0.717–0.901). This places the probability of the patient being classified correctly as asthmatic at 83.3%. The cut-off level for FeNO to screen for asthma was taken as 19.45 at 71.2% sensitivity and 81.8% specificity ($p < 0.001$) (Fig. 1). The positive predictive value 96.84% (95% CI: 94.43–98.23) and negative predictive value 30% (95% CI: 23.78–37.05) for asthma prediction with FeNO.

In determining the diagnostic accuracy of FeNO for atopy, the AUC for FeNO value was 0.648 (95% CI, 0.599–0.696). This places the probability of the patient being classified correctly as SPT positive at 64.2%. The cut-off level for FeNO to screen for atopy was not taken, as it is not a good predictor ($p = 0.001$) (Fig. 2).

In establishing the diagnostic accuracy of FeNO for atopy among asthmatics and HC, the FeNO values were [0.662 (95% CI, 0.610–0.714) vs. 0.421 (95% CI, 0.281–0.562)]. The cut-off level for FeNO to screen for atopy among asthmatics and non-asthmatics were not taken, as it is not a good predictor ($p = 0.001$) (Fig. 3A, B).

Discussion

The FeNO level measurement has been validated and standardized for supporting the diagnosis in cases of eosinophilic inflammation of the airways, bronchial hyper-reactivity, and asthma [5].

A number of host factors were associated with FeNO levels. The most important factors were age, gender, race/ethnicity, and atopy. The normal range of FeNO levels varies from studies in a variety of countries considering the effects of these factors. The FeNO range in HC in a study from India was 12.73 ± 7.8 ppb, whereas other studies of the Chinese, African population, and data from asthma, and allergy research group had the mean FeNO levels ranging from 20 to 39ppb [12, 24–28]. The present study had FeNO levels of 12.91 ± 10.35 ppb.

ATS/ERS guidelines state that there is an age-dependent increase in FeNO levels in children ≤ 12 years of age [20], but there is less agreement across the studies regarding the relationship with the age of adults. The present study did not find correlation with age, which is consistent with the literature. FeNO interacted significantly with

Table 1. Clinical and spirometric characteristics of the study population

	Bronchial Asthma (n = 215)	Bronchial asthma with allergic rhinitis (n = 248)	Healthy controls (n = 65)
Age, Mean ± SD	26.7 ± 8.5	25.9 ± 8.6	28.2 ± 6.1
Gender			
Female, Freq (col %)	112 (52.1%)	112 (45.2%)	27 (41.5%)
Male, Freq (col %)	103 (47.9%)	136 (54.8%)	38 (58.5%)
Symptoms			
Breathlessness, Freq (col %)	200 (93.0%)	237 (95.6%)	–
Wheeze, Freq (col %)	193 (89.8%)	220 (88.7%)	–
Cough, Freq (col %)	195 (90.7%)	219 (88.3%)	–
A.E.C.			
Mean ± SD	509.0 ± 298.8	506.9 ± 270.7	127.8 ± 85.8
Median (IQR)	500 (400)	500 (395)	100 (130)
S.Total IgE			
Mean ± SD	538.2 ± 414.1	681.2 ± 572.5	113.0 ± 68.7
Median (IQR)	487 (430)	579 (503)	105 (98)
SPT Positive, Freq (col %)	116 (54.0%)	149 (60.1%)	30 (46.2%)
No. of allergens in SPT Positive patients, Mean ± SD	4.8 ± 8.2	3.8 ± 4.9	1.6 ± 2.5
FeNO Value, Mean ± SD	32.2 ± 26.4	33.8 ± 23.1	12.9 ± 10.3
FeNO			
< 19.45, Freq (col %)	62 (28.8%)	64 (25.8%)	54 (83.1%)
> 19.45, Freq (col %)	153 (71.2%)	184 (74.2%)	11 (16.9%)
FEV_1(obs)% , Mean ± SD	83.3 ± 19.2	83.9 ± 16.9	95.7 ± 9.7
FVC(obs)% , Mean ± SD	92.8 ± 15.8	90.9 ± 14.2	100.8 ± 10.5
FEV_1/FVC(pred)% , Mean ± SD	77.7 ± 12.9	79.3 ± 12.7	90.3 ± 8.8
Reversibility, Freq (col %)	55 (25.6%)	66 (26.6%)	–
Severity of airflow limitation			
No, Freq (col %)	118 (54.9%)	127 (51.2%)	59 (90.8%)
Mild, Freq (col %)	48 (22.3%)	70 (28.2%)	6 (9.2%)
Moderate, Freq (col %)	24 (11.2%)	34 (13.7%)	–
Moderately Severe, Freq (col %)	14 (6.5%)	9 (3.6%)	–
Severe, Freq (col %)	8 (3.7%)	6 (2.4%)	–
Very Severe, Freq (col %)	3 (1.4%)	2 (0.8%)	–

BMI — body mass index; S.T.IgE — serum total immunoglobulin E; AEC — absolute eosinophil count; FeNO — fraction of exhaled nitric oxide; NS — not significant

height in children and in adults, because of the increased lung size with taller height [29].

The present study did not reveal a significant association of BMI with FeNO. However, Maniscalco *et al.* [30] observed a reduction in FeNO in 24 adults with severe obesity, which was restored after weight loss. Men have consistently higher FeNO levels as compared to women [5]; even the present study reports higher FeNO levels in men. Spergel *et al.* studied the correlation between FeNO and monthly symptom scores and documented significant correlation ($r = 0.646$ and $r^2 = 0.417$; $p < 0.0001$); FeNO was also able to distinguish between mild and moderate to severe persistent asthmatic patients [15]. However, the present study did not find any significant correlation of FeNO with duration of symptoms. In a study of 222 asthmatics, and 27 HC by Banovicin *et al.* [15], subjects with positive family history for allergic diseases (bronchial asthma, allergic rhinitis/rhino conjunctivitis, atopic eczema) showed higher FeNO (27. 8 ± 2.8 vs. 20.1 ± 1.8 ppb, p

Table 2. Correlation of FeNO values (categorized at cut-off of 19.45) with age, clinical and spirometric characteristics

Characteristic	Odds ratio	95% CI		p
		Lower	Upper	
Age	0.975	0.949	1.003	0.077
Gender	0.566	0.361	0.887	0.013
BMI (kg/m^2)	0.976	0.932	1.021	0.294
Duration of symptoms (years)	1.036	1.011	1.061	0.004
Breathlessness	9.357	2.418	36.214	0.001
Wheeze	1.132	0.471	2.718	0.782
Cough	0.669	0.246	1.821	0.431
Family history	1.637	1.112	2.411	0.013
AEC	1.002	1.001	1.003	0.001
S.Total IgE	1.000	1.000	1.000	0.940
SPT Positive	3.555	1.870	6.758	< 0.001
NO of SPT positive	1.039	0.968	1.115	0.293
FEV_1	1.000	0.971	1.030	0.991
FVC	1.006	0.981	1.031	0.643
FEV_1/FVC	0.991	0.962	1.020	0.533
Reversibility	0.743	0.390	1.413	0.365
Severity of airflow limitation	0.984	0.694	1.396	0.929

BMI — body mass index; S.T.IgE — serum total immunoglobulin E; AEC — absolute eosinophil count; FeNO — fraction of exhaled nitric oxide; FEV_1 — forced expiratory volume in 1 second; FVC — forced vital capacity

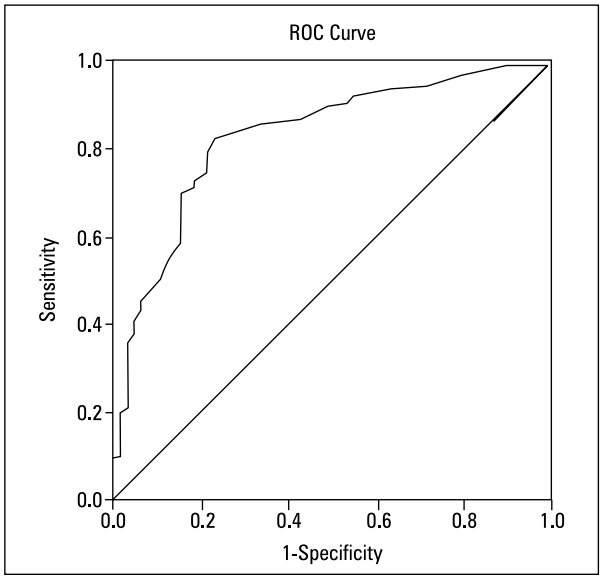

Figure 1. FeNO values to predict the diagnosis of asthma

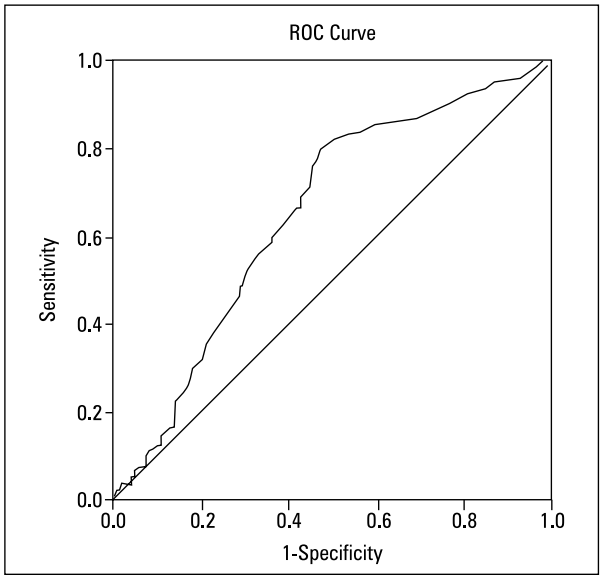

Figure 2. To determine the diagnostic accuracy of FeNO as a predictor of atopy

= 0.067). The same study also reported elevated FeNO, which was associated with higher total serum IgE levels and eosinophilia. The present study confirms the positive correlation of FeNO with family history of asthma, and eosinophilia. It did not show association with S.T.IgE levels.

Atopy is a clinical definition of an IgE-antibody responder, which is a personal tendency to become sensitized and produce IgE antibodies in response to allergens.

A strong association between FeNO and atopy has been documented in the recent literature

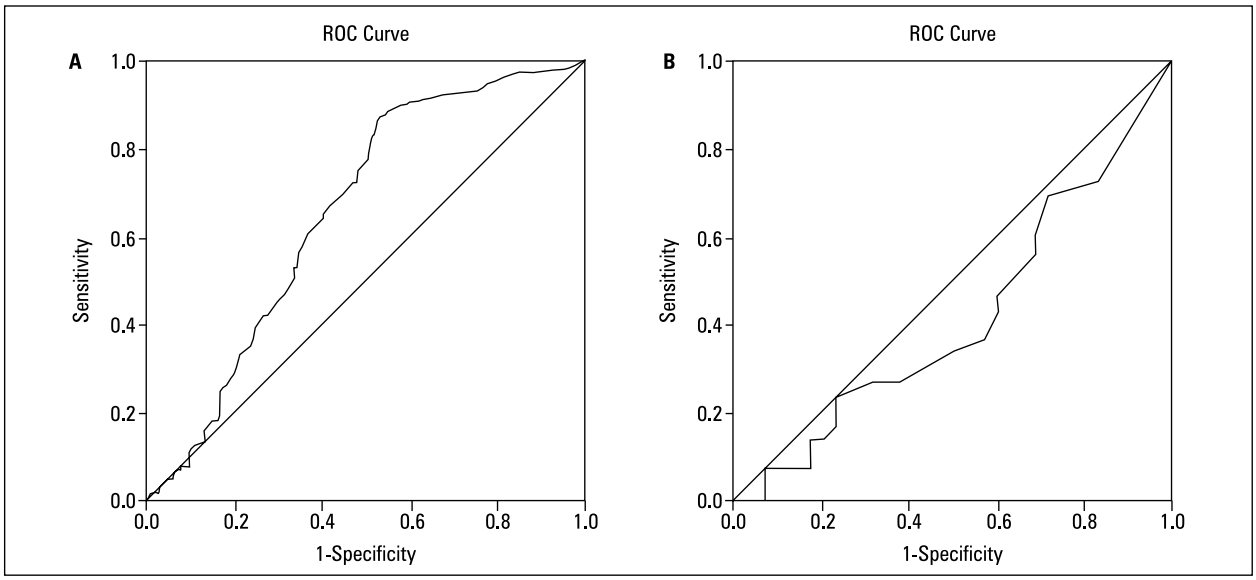

Figure 3. A — to determine the diagnostic accuracy of FeNO as a predictor of atopy among asthma subjects; **B** — to determine the diagnostic accuracy of FeNO as a predictor of atopy among healthy controls

[6, 12, 31]. Also, the association between the degree of atopy and FeNO levels has been reported [12, 31, 32], with the conclusion that, as the number of positive responses to skin prick test increases, the FeNO levels also increase [6, 12, 33]. This finding has been attributed to the difference in inflammatory cell recruitment in atopic (eosinophilic) and non-atopic asthmatics (neutrophilic), as well as to cell activity of NO-producing cells [28]. In accordance with the literature, the present study documented a positive correlation of FeNO with atopy. However, FeNO was not a good predictor of atopy among asthmatic and non-asthmatic subjects.

Asthma symptoms are typically caused by airflow limitation caused by a narrowed airway. FeNO measures airway inflammation that can produce changes in the lining of the airway, which may or may not result in asthma symptoms and turbulent airflow from obstruction. FeNO and FEV_1, therefore, measure different components of the asthmatic physiology. In the literature, FeNO correlates only with the spirometry parameters relating to airflow obstruction present in the small airways. Delguidice *et al.* [34], reported significant correlations between FeNO and FEV_1 ($p < 0.0059$, $r = 0.468$), and between FeNO and FEF25–75 ($p < 0.0098$, $r = 0.439$). Similarly, Stănciulescu *et al.* [17], outlined relationship between FeNO and MEF25–75 [17]. In the present study, FeNO did not show significant negative associations with FEV_1 and FEV_1/FVC. In like manner, in a study by Spergel *et al.* [16], FeNO did not correlate with FEV_1 reflecting the fact that these 2 parameters measure different aspects of asthma.

The limitation of the present study was that the exposure to allergen at the time of FeNO measurement was not documented. Also, a larger number of control subjects could have been recruited for establishment of factors affecting FeNO measurement.

Conclusion

The study highlights the importance of estimation of anthropometric parameters and dyspnea assessment in the evaluation of FeNO levels. Also, the presence of atopy may influence the results in the interpretation of FeNO readings. Furthermore, the study has demonstrated that spirometry and FeNO have no significant correlation, which further lays emphasis on them as being different physiological parameters of asthma.

Conflict of interest

The authors declare no conflict of interest.

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