Measurement of exhaled nitric oxide in healthy Egyptian population: normal ranges and factors affecting

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Background Nitric oxide is an important regulatory mediator throughout the body. Naturally, the diagnostic applicability of fraction of exhaled nitric oxide (FeNO) depends on the availability of reference values that adequately take into account the major factors affecting FeNO. FeNO values are strongly influenced by several intraindividual factors, including age, atopy, high immunoglobulin E, height, weight, sex, and smoking habits. This study aimed to address the normal ranges of FeNO in healthy Egyptian adults and its relation to other personal factors.

Materials and methods A total of 211 healthy Egyptian individuals were selected from pulmonary outpatient clinics and the Chest Department of University Hospital during the period between January 2014 and September 2014. Pulmonary function tests, FeNO measurement, and laboratory tests were carried out. The participants' demographic data were also recorded.

Results There was significant negative correlation between measured FeNO and age, weight, BMI, and smoking index. A positive correlation was found between FeNO and height. Female participants had significantly lower levels of FeNO (20.4 ± 9.9) compared with male nonsmokers (28.2 ± 12.4).

Conclusion FeNO is affected by sex, BMI, weight, height, and current smoking. The reference ranges for FeNO in healthy Egyptian adults were similar to those of the Caucasian population. In general, values of more than 50 parts per billion (ppb) in male participants and 40 ppb in female participants are considered abnormal in Egyptian populations. *Egypt J Broncho* 2015 9:48–54

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Background

Nitric oxide (NO) is an important regulatory mediator throughout the body. Its measurement in exhaled air [fractional exhaled nitric oxide (FeNO)] is useful in disease monitoring in asthma, and in the diagnosis of congenital ciliary dyskinesias [1]. Endogenous NO is a gaseous signaling molecule produced by residential and inflammatory cells in both large and peripheral airways and alveoli. NO plays an important role in regulating airway and vascular function and is generated by three isoforms of NO synthases (NOS) [neuronal NOS (nNOS, NOS1), endothelial NOS (eNOS, NOS3), and inducible NOS (iNOS, NOS2)] with different expression and pathophysiologic roles in the airways. In particular, iNOS is not constitutively expressed but is induced by several stimuli including endogenous mediators (chemokines and cytokines) and exogenous factors (bacterial toxins, viral infection, allergens, environmental pollutants, etc.) [2]. NO is generated by the conversion of amino acid L-arginine to amino acid L-citrulline and during inflammation, due to iNOS induction, large amounts of NO are produced, which may exert proinflammatory effects [3]. The measurement of the FeNO is recognized as an accurate, reproducible, and completely noninvasive diagnostic test for airway disease [4]. FeNO can be detected in exhaled air by several methods such as chemiluminescence, spectroscopy, electrochemical portable, and other methods currently under development [5].

Naturally, the diagnostic applicability of FeNO depends on the availability of reference values that adequately take into account the major factors affecting FeNO. In the last few years, a number of publications have provided such reference values on the basis of population samples of adults or children [6–11]. FeNO values are strongly influenced by several intraindividual factors, including age [12,13], atopy [14,15], high immunoglobulin E [16], height, weight, sex, and smoking habits [17]. In general, the numeric value of a diagnostic test can be presented in several forms: the absolute value, the percentage of predicted of a reference value, and *Z*-scores. Absolute values are presently used in the interpretation of FeNO, but the percentages of predicted values are used for the interpretation of lung function tests [18]. It has been proposed that a 'personal best' value for FeNO might be used [19]. The measurement of FeNO is increasingly recognized as an important addition to pulmonary function testing in clinical practice [20,21]. As FeNO measurement is an important marker in the assessment of airway inflammation and

is of potential use in clinical practice, there is a need to establish a reference for different populations to aid in the interpretation of measured values. Furthermore, ATS/ERS has encouraged investigators to publish physiological normal values for healthy populations of various racial backgrounds to enable an individual's results to be compared with data from a racially similar population [4]. In the Arab population, there are only two studies that have been published on normal values of FeNO [22,23].

The aim of this study was to identify factors that influence the FeNO values of healthy Egyptian adults in different age groups, as well as establish a reference range for FeNO in Egyptians and study its applicability and reliability in similar populations compared with other studies conducted in the nearby countries and other values published worldwide.

Materials and methods

Study population and design

This study was carried out in pulmonary outpatient clinic in association with chest department of university hospital in the period between January and September 2014 and it was approved by the local ethics committee. A total of 300 healthy adults from the community were invited to participate in this study, but only 211 accepted to participate, whereas 89 refused. All individuals who accepted to participate in this study were included, and a written informed consent was obtained from each person. Individuals who refused to participate in this study were excluded. Other exclusion criteria included a history or manifestations of atopic diseases (such as allergic asthma, atopic dermatitis, allergic rhinitis, and food allergy), acute respiratory tract infection in the last 4 weeks, and chronic respiratory illness (such as chronic obstructive pulmonary disease, interstitial lung disease, and bronchiectasis, etc.). Individuals who had obstructive abnormality in spirometry defined by forced expiratory volume in first second/forced vital capacity (FEV_1/FVC) less than 0.7 or those on current use of inhaled or systemic steroid were also excluded. The participants were asked to answer structured questionnaires on medical history and allergic symptoms. A thorough clinical examination, chest radiography, spirometry, and other laboratory tests were performed. Individuals who fulfilled the inclusion criteria underwent FeNO.

Fraction of exhaled NO measurement

FeNO was measured using an online electrochemical nitric oxide monitor (NIOX MINO; Aerocrine, AB Solna, Sweden) according to 2005 ATS/ERS guidelines [4], with a sensitivity of one parts per billion (ppb). This nitric oxide analyzer has been approved by the US Food and Drug Administration for clinical use. The participants were asked about current medication or food intake that could interfere with the FeNO measurement results. In addition, they were instructed to avoid smoking, exercise, and ingestion of food, water, or caffeine at least 4 h before testing. All tests were performed at the same time of the day between 13 : 00 and 16: 00 h daily to minimize possible circadian effects. The procedure was started by asking the individuals to exhale completely to empty their lungs outside the analyzer and then to inhale to total lung capacity through the mouthpiece and finally exhale into the device at a constant expiratory flow rate of 50 ml/s (±10%) over 10 s and a pressure of 10 cmH_2O according to the guideline recommendation [4]. Three acceptable and reproducible maneuvers (within 10% deviation) were performed. Final FeNO values were calculated as the arithmetic mean of these values. The NO analyzer was calibrated every 2 weeks using a certified calibration gas (Linde, Munich, Germany) according to the manufacturer's recommendations. Ambient air pressure, temperature, humidity, and ambient NO levels were recorded for each measurement.

Pulmonary function tests

Before performing the test, ambient temperature and pressure were entered along with the patient data [age (years), weight (kg), height (cm), and sex] so that all results were calculated as percent-of-predicted (% predicted) except for FEV_1/FVC. Pulmonary function tests were performed using a Sensor-medics Vmax series, 2130 spirometer, V 6200 Autobox, 6200 DL (Sensor Medics Corporation, California, USA). Flow/volume loop was performed to all participants. Individuals with FEV_1/FVC less than 0.7 and FEV_1 less than 80% of predicted were excluded from the study. All individuals who were included in this study had to have normal spirometry.

Other laboratory tests

Complete blood count was performed to exclude acute infection and eosinophilia. Random blood sugar, liver function tests, and kidney function tests were performed to exclude chronic illness.

Statistical analysis

The data were analyzed using the statistical package for social sciences, version 11. Data were expressed as mean ± SD for continuous variables and as percentages for categorical variables. Comparisons between variables were performed using Student's *t*-test for continuous variables. Frequency distribution and cumulative percentage were determined for FeNO.

We calculated Pearson's correlation coefficient to see the relation of FeNO with other parameters. Multiple linear regressions were carried out to find the predictor variables for FeNO. A *P*-value of 0.05 or less was considered statistically significant and all tests were two tailed.

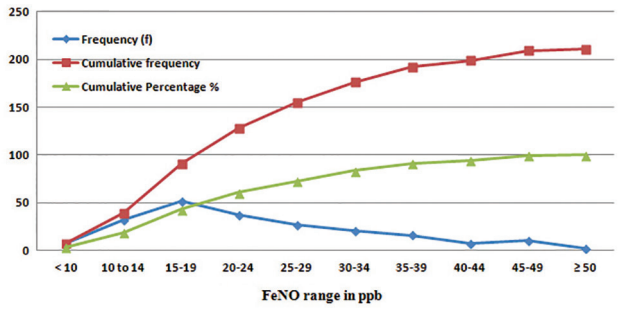
Results

Baseline characteristics

Baseline demographic characteristics are presented in Table 1 with pulmonary functions tests and laboratory data. The study included 211 healthy Egyptian individuals (155 men and 56 women). There were 47 male participants who were smokers, whereas all female participants were nonsmokers. The mean age of men was 37.6 ± 13.1 and the mean age of women was 37 ± 14.1. The mean BMI in men was 30.2 ± 6.3 and the mean BMI in women was 31.4 ± 7.4. Pulmonary function tests, white blood cell, eosinophil count were all within normal values. Fraction of FeNO is also demonstrated in Table 1. The measured FeNO ranged between 6 and 50 ppb, with mean value of 25.5 ± 11.6 in men (28.2 ± 12.4 in nonsmokers and 19.4 ± 8.2 in smokers). In women the mean value of FeNO was 20.4 ± 9.9.

The cumulative percentage distribution based on different ranges of FeNO is shown in Table 2, and the distribution of FeNO based on different ranges of FeNO is shown in Fig. 1. There was a significant negative correlation between measured FeNO and age, weight, BMI, and smoking index (Table 3 and Fig. 2) but a positive correlation between FeNO and height was observed in all groups.

Fig. 1



Cumulative percentage distribution based on different ranges of FeNO. FeNO, fractional exhaled nitric oxide; ppb, parts per billion.

Table 2 Cumulative percentage distribution based on different ranges of FeNO

FeNO (ppb)	Frequency (*f*)	Cumulative frequency	Cumulative percentage
<10	7	7	3.32
10–14	32	39	18.48
15–19	52	91	43.1
20–24	37	128	60.7
25–29	27	155	73.0
30–34	21	176	83.4
35–39	16	192	91.0
40–44	7	199	94.3
45–49	10	209	99.1
≥50	2	211	100

FeNO, fractional exhaled nitric oxide; ppb, parts per billion.

Table 1 Demographic characteristics, pulmonary function tests, and laboratory data of all participants

Variables	Total number of men (*N* = 155)	Male nonsmokers (*N* = 108)	Male smokers (*N* = 47)	Women (*N* = 56)	Total number of participants (*N* = 211)
Age (years)					
Mean ± SD	37.6 ± 13.1	38.6 ± 13.4	34.7 ± 11.7	37 ± 14.1	37.4 ± 13.4
Weight (kg)					
Mean ± SD	86.7 ± 19.6	86.1 ± 19.8	88.1 ± 19.4	76.1 ± 19	83.9 ± 19.9
Height (cm)					
Mean ± SD	170 ± 7.5	168.8 ± 7.3	172.6 ± 7.5	155.5 ± 6.6	166.2 ± 9.7
BMI (kg/m^2)					
Mean ± SD	30.2 ± 6.3	30.6 ± 6.9	29.8 ± 5.1	31.4 ± 7.4	30.5 ± 6.6
Smoking index					
Mean ± SD	61.7 ± 169.2	0	203.5 ± 515.1	0	45 ± 17.4
FEV_1/FVC					
Mean ± SD	88.6 ± 6.9	90.4 ± 8.4	82.6 ± 5.7	90 ± 7.9	88.9 ± 7.2
FEV_1					
Mean ± SD	85.2 ± 18.6	88.7 ± 14.5	80.3 ± 4.9	88.4 ± 13.4	86.1 ± 17.4
FeNO (ppb)					
Mean ± SD	25.5 ± 11.6	28.2 ± 12.4	19.4 ± 8.2	20.4 ± 9.9	24.1 ± 11.4
WBCs					
Mean ± SD	7.4 ± 2.0	6.4 ± 3.1	9.2 ± 2.4	8.0 ± 1.8	7.6 ± 2
Eosinophil count					
Mean ± SD	2.3 ± 1.2	2.9 ± 1.4	1.7 ± 0.9	1.7 ± 0.97	2.2 ± 1.2

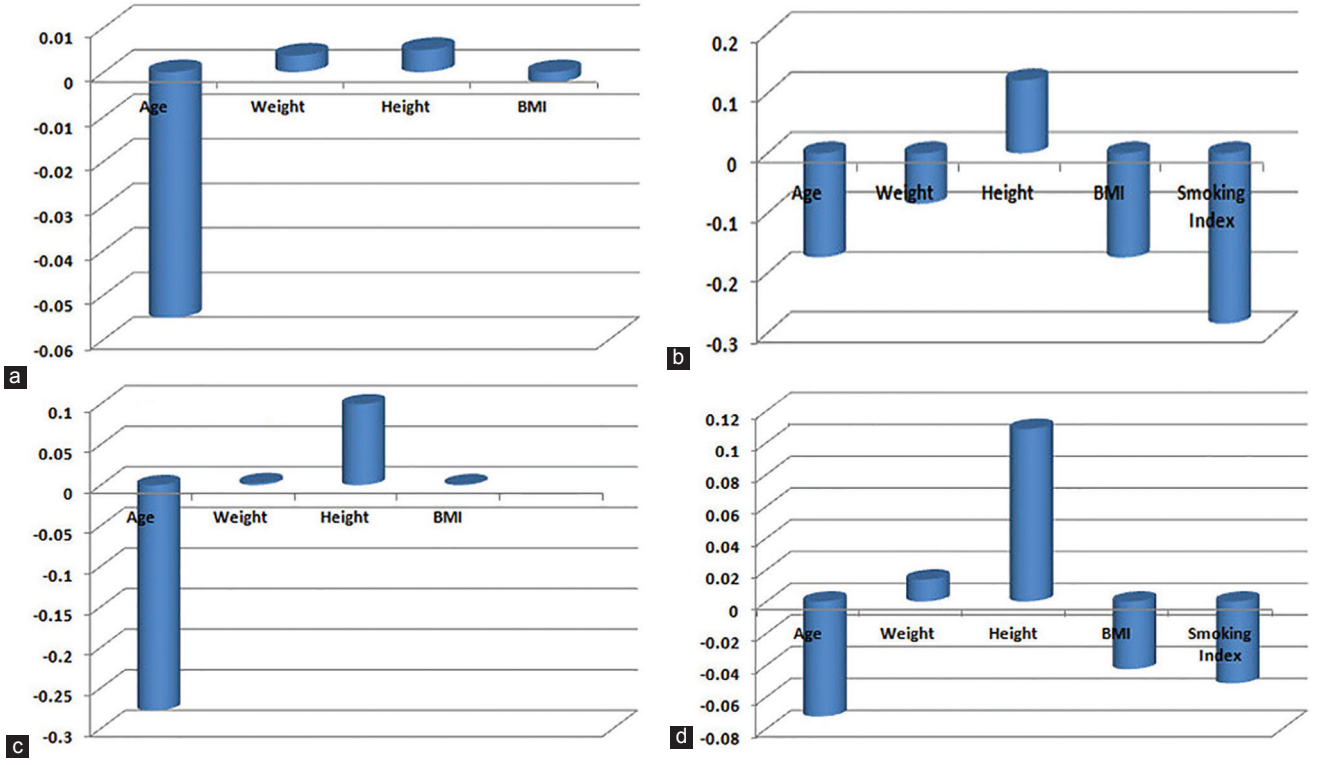
FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; ppb, parts per billion; WBC, white blood cell.

Table 3 Correlation coefficient between FeNO and other variables

Variables	FeNO							
	Male nonsmokers (*N* = 108)		Male smokers (*N* = 47)		Women (*N* = 56)		Total number of participants (*N* = 211)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age	−0.0551	0.0002	−0.1738	0.0428	−0.2774	0.0006	−0.0723	0.1275
Weight	−0.00373	0.1763	−0.0844	0.0014	−0.0025	0.0001	−0.0138	<0.00001
Height	0.00497	0.0005	0.1219	0.0002	0.0996	0.000002	0.1087	0.0094
BMI	−0.00238	0.000001	−0.1742	0.0236	−0.0012	0.0023	−0.0426	<0.00001
Smoking index	–	–	−0.2843	0.0001	–	–	−0.0515	0.9843

FeNO, fractional exhaled nitric oxide; $P \leq 0.05$ = significant; $P \leq 0.005$ = highly significant.

Fig. 2



Correlation coefficient between FeNO and other variables. Correlation coefficient between FeNO and other variables in male nonsmokers (a). Correlation coefficient between FeNO and other variables in male smokers (b). Correlation coefficient between FeNO and other variables in females (c). Correlation coefficient between FeNO and other variables in all subjects (d). Correlation coefficient of FeNO with all variables in all groups (e). FeNO, fractional exhaled nitric oxide.

Discussion

Exhaled NO originates primarily in the airway epithelium, produced by inducible NO synthetase [17]. Because biological NO formation is a complex and energy-consuming process, airway NO formation is important in humans and, consequently, should be under tight biological control under normal circumstances. Its origin from the airway epithelium indicates that the total surface area of the airway mucosa will be an important determinant for exhaled NO [18]. Thus, it is logical that age and BMI were found to be important factors when evaluating FeNO values, as seen for other lung function parameters. In addition to individual-specific factors that affect the value of FeNO, several behavioral and environmental factors have been pointed out as influencing FeNO, such as allergen exposure [24], physical exercise [25,26], ozone exposure [27,28], and air pollution [29].

This study was carried out on 211 healthy Egyptian individuals, and results showed that FeNO values were significantly higher in men (25.5 ± 11.6 ppb) than in women (20.4 ± 9.9 ppb). These results matched with previous studies [30–34] in that the mean values of FeNO were higher in men than in women, but their measurements of FeNO were at lower levels (18.2 ± 10.6 in men and 12.1 ± 6.9 in women) than those in this study. This difference can be explained by the effect of environmental changes; all these studies were

conducted in the Far East region, whereas this study was conducted in the Caucasian middle east region. Moreover, those studies investigated populations with means of ages and BMI different than those in the current study.

For comparing the effect of sex on FeNO values in some of our areas, there were two studies conducted in nearby areas: one in Saudi Arabia and another in Tunisia. The study conducted in Kingdom of Saudi Arabia investigated only the FeNO in men and found the mean of FeNO to be 22.79 ± 8.13 ppb, which is close to that found in our study [23]. In Tunisia, the mean value of FeNO was at lower level compared with the current study and with no male-to-female differences (13.31 ± 4.55 in men and 13.84 ± 5.26 in women) [22]. Other studies conducted in different regions were in agreement with our study and showed higher level of FeNO in men than in women [9,11,20,35]. The sex-related differences of FeNO were explained by differences in the surface area of the airway epithelium, the major source of exhaled NO, and for which height is an important anthropometric correlate [20]. Moreover, genetic and hormonal factors also have a role in FeNO levels [36]. In fact, estrogen can activate the endothelial NO synthetase in human bronchiolar epithelial cells *in vitro* [37]. Moreover, relationship between estrogen/progesterone levels and exhaled NO has recently been described [38].

As regards age, this study included individuals with age ranging between 18 and 60 years. The results showed that there was negative correlation between the mean of FeNO and age, with highly significant differences. This finding is accepted in adult population with ages between 18 and 60 years, but in children and elderly more than 60 years there were some differences. In the elderly, Gelb *et al*. [39] recently showed that FeNO and alveolar NO, but not bronchial NO flux, increased with age in adults, especially in those above 60 years. This is suggested to be because of the reduced lung diffusing capacity for NO (and carbon monoxide) seen in elderly, leading to less uptake of bronchial NO in the alveolar tract. There have been conflicting reports regarding the association between FeNO and age [32,40]. Potentially, this relationship is only detectable at very young ages and in advanced age, and it might be negligible during young adulthood. Possible explanations for an increase in FeNO with age could include diet [41], latent respiratory inflammation, or an increase in peripheral (alveolar) NO [39].

The present study identified a negative correlation of both weight and BMI with FeNO. This finding was supported by the findings of Maniscalco *et al*. [42] who observed that FENO is consistently reduced in severe obesity, and it is restored after weight reduction. This was explained by the effect of obesity on systemic oxidative stress, in part through increased production of reactive oxygen species in adipose tissue. It is hypothesized that the lung serves as a target organ for this oxidative stress. This is manifested as increased oxidation of airway NO into nitrate and reactive nitrogen species and hence the reduction of NO bioavailability and exhaled NO levels [23]. Some other studies showed no significant relationships between BMI and FeNO values [43–46].

Many studies investigated the effects of smoking on FeNO values in adults. There was consistent evidence that active smoking and acute cigarette smoke exposure lead to a transient decrease in FeNO levels in healthy and asthmatic adults [47,48]. In current study, there was a significant negative correlation between FeNO and smoking index with lower levels of FeNO in current smokers than in nonsmokers, which matched with the previous studies [47–49]. A record of smoking history is therefore necessary for the interpretation of results, and measurement is advised at least an hour after smoking.

As regards height, it is the only parameter that is positively correlated with FeNO in normal healthy participants. Olin *et al*. [40] noted that FeNO was clearly positively correlated with height in both men and women, which matched with the current study. This is probably because of the height-dependent increase in the total airway mucosal surface area that produces NO [50]. Therefore, height is the strongest independent predictor [51] for FeNO measurement.

Conclusion

FeNO is affected by sex, BMI, weight, height, and current smoking. In healthy Egyptian adults, women had lower levels of FeNO compared with male nonsmokers. In general, values of more than 50 ppb in men and 40 ppb in women are considered abnormal in Egyptian populations. FeNO measurement is a noninvasive tool, easy to perform, and it may add benefits in diagnostic tests in pulmonary diseases.

Acknowledgements

Conflicts of interest

None declared.

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