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Fraction of exhaled nitric oxide in healthy elderly Tunisian subjects☆

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ABSTRACT

Introduction: Exhaled-fraction-of-nitric-oxide (Fe_{NO}) norms are absent in healthy elderly North-African subjects.
Objectives: i) to identify Fe_{NO} influencing factors of elderly Tunisians older 50 years and more; ii) to assess the applicability of some published Fe_{NO} norms for elderly in local population; iii) to set-up Fe_{NO} norms and to prospectively evaluate their validity in two elderly validation-groups (healthy and asthmatic subjects).
Methods: A convenience sample of healthy and asthmatic elderly Tunisians was recruited. Subjects responded to a medical questionnaire and then Fe_{NO} levels were measured by an online method (Medisoft, Sorinnes (Dinant), Belgium). Clinical, anthropometric and spirometric data were collected. Three groups of subjects were identified: group I (healthy-elderly; n = 100, 57 females); group II (healthy-validation; n = 17, 4 females) and group III (asthmatic-validation; n = 10, 9 females). ANOVA was performed to compare the three groups' data.
Results: No significant factor, among those evaluated, influenced Tunisian elderly Fe_{NO} values. The available published Fe_{NO} norms did not reliably predict Fe_{NO} in Tunisian elderly population. The mean ± SD (minimum–maximum) of Fe_{NO} (ppb) for group I was 14 ± 6 (3–34). For Tunisian people, each elderly Fe_{NO} value higher than 34 ppb will be considered as abnormal. There was no statistical significant difference between Fe_{NO} (ppb) mean values of group I and groups II (15 ± 8) or III (18 ± 13). No subject of group II had a Fe_{NO} value higher than 34 ppb. Thirty percent of group III subjects had a Fe_{NO} value higher than 34 ppb.
Conclusion: In practice, Fe_{NO} value of more than 34 ppb is considered abnormal in elderly Tunisian population.

1. Introduction

Asthma is one of the most frequent chronic respiratory diseases [1]. Its prevalence in elderly North-African population was 6.5% in 2009 [1]. During the process of the chronic airway inflammation [2], *nitric oxide* (NO) synthesis is increased by eosinophilic infiltration [3]. For that reason, measurement of *fractional exhaled NO* (Fe_{NO}) concentration has been proposed as a useful biomarker for monitoring and management of airway diseases [3]. Fe_{NO} can be used to diagnose asthma [4], predict responses to corticosteroid treatment [5], detect early loss of control [6] and tailor inhaled corticosteroid medication [7].

General population studies, performed in young and middle-aged adults, have reported that Fe_{NO} levels are influenced by various demographic and clinical factors such as sex [8–12], age [10,11,13], height [8,10–13], atopy [8–10,12,13], smoking status

List of abbreviations: ANOVA, Analysis-of-variance; ATS, American-thoracic-society; BMI, Body-mass-index; ERS, European-respiratory-society; $FEF_{x\%}$, forced-expiratory-flow when x% of FVC has been exhaled; Fe_{NO}, Fraction-of-exhaled nitric-oxide; FEV_1, 1st second-forced-expiratory-volume; FMEF, forced-mid-expiratory-flow; FVC, Forced-vital-capacity; IgE, Immunoglobulin E; LLN, Lower-limit-of-normal; LOA, Limits-of-agreement; n, number; NO, Nitric oxide; PEF, Peak-expiratory-flow; ppb, Parts-per-billion; r^2, Determination-coefficient; r, Correlation coefficient; SD, Standard-deviation; ULN, Upper-limit-of-normal; 95%CI, 95% confidence-interval.

☆ The French abstract of the present study was previously presented as a poster at the Mediterranean Forum of Geriatrics (November 2014, Tunisia, Sousse) and Medical Days of the Center (December 2014, Tunisia, Sousse). The English abstract of the present paper is accepted as a poster in the upcoming congress of the European Respiratory Society (Amsterdam, September 2015). First author presenting such data: Ines SFAXI.

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[11,12] and racial differences [11]. These findings suggest that Fe_{NO} determining factors should be identified for each racial population [9]. However, in healthy elderly older 50 years and more, and at the best of the authors' knowledge, only one study [8] has analyzed the Fe_{NO} influencing factors.

Interpretation of Fe_{NO} data relies upon comparison of measured values with predicted ones obtainable from published norms (e.g., fixed values, reference equations or normal values tables) [14–16]. In North–Africa, Fe_{NO} norms were previously developed for healthy Tunisian Arab aged 6–60 years [17,18]. However, no local Fe_{NO} norms exist for elderly populations and those derived from other populations [8–14,19–21] are commonly used. This is a questionable practice. On the one hand, their applicability and reliability have never been verified in Tunisia. On another hand, their use may lead to erroneous clinical interpretation of Fe_{NO} data. Norms should be obtained from studies of "normal" or "healthy" subjects with the same anthropometric and, where relevant, ethnic characteristics of the patient being tested [22]. The applicability and reliability of these published norms [8–13,19,21] should be evaluated as regards to Tunisian elderly. Moreover, the *American-thoracic* and the *European-respiratory societies* (ATS/ERS) have encouraged investigators to circulate physiological norms for healthy populations of different racial backgrounds to permit individual subject results to be compared with data from a racially comparable population [14]. The use of analogous type of assessment equipment and method was also suggested [14].

Therefore, the present study aims:

i) To identify factors that influence the Fe_{NO} values of healthy elderly Tunisian subjects older 50 years and more,
ii) To test the applicability and reliability of the previously published Fe_{NO} reference equations [10,11,13,19] or normal values [8,9,12,21] in this population (the null hypothesis is that there will be no difference between measured and predicted Fe_{NO} mean values or no healthy elderly Tunisian subject with a higher Fe_{NO} value than the published thresholds),
iii) And, if needed, to set up Fe_{NO} norms for healthy elderly Tunisian subjects and to prospectively evaluate their reliability.

2. Methods

2.1. Study design

It is a cross-sectional study spread over nine months (April–December 2014). It was conducted at the Department of Physiology and Functional Explorations (Farhat HACHED Hospital, Sousse, Tunisia).

Study design consists of a convenience sample of healthy subjects older 50 years and more living in the region of Sousse, Tunisia.

The study was conducted in compliance with the 'Ethical principles for medical research involving Human subjects' of the Helsinki Declaration (available from: http://www.wma.net/en/30publications/30ethicsmanual/pdf/ethics_manual_arabic.pdf, accessed 24th June 2015). Approval for the study was obtained from the Hospital ethics committee (Farhat HACHED Hospital ethics committee, approval number 2801/2013). Written informed consent was obtained from all subjects.

2.2. Sample size

It was calculated according to the following equation [23]: $n = (Z^2\ p\ q)/\Delta^2$, where "n" was the number of required elderly subjects, "Z" was the 95% confidence level (=1.96), "q" was equal to "1-p", "Δ" was the precision (=6%), and "p" was the current proportion of healthy people older 50 years and more in Tunisia (10.1% in 2011, available from: http://www.ins.nat.tn/indexfr.php, accessed 24th June 2015). Plugging this appropriate value into the equation, the sample size was thus 97 subjects. Therefore, to establish Fe_{NO} norms, an initial healthy-group of 100 elderly subjects (57 females) was recruited.

2.3. Subjects

Three groups were recruited: healthy-norms group (group I), healthy and asthmatic validation-groups (respectively, groups II and III).

2.3.1. Group I (healthy-norms-group)

Volunteer healthy subjects older 50 years and more were included. Subjects were recruited among parents of Hospital workers and/or of medical school students and among elderly people residing in a retirement home. In addition, an article announcing the need for recruitment of elderly healthy subjects was posted on a social network service (Facebook page of IS). The following non-inclusion criteria, similar to a local previous study [18], were applied: fever, chronic diseases (cardiovascular, renal, gastrointestinal, neurological; otorhinolaryngologic), allergic rhinitis, recurrent symptoms or rhinitis, symptoms and signs of acute upper respiratory infection during three weeks prior to assessment, recent airway infection (cold, flu, sore throat within the last seven days); clinical manifestation of allergic diseases (urticaria, skin allergy, atopic dermatitis, eczema); history of pulmonary diseases or related respiratory symptoms (asthma or asthma medication use, current or past symptoms of wheeze or chronic cough and chronic obstructive pulmonary disease), regular medication (glucocorticoid, bronchodilator, leukotriene receptor agonist, antihistamine, etc.) and current or ex-smoker (cigarettes or narghile use) [24]. Exclusion-criteria were: obstructive-ventilatory-defect, abnormal spirometric data, inability to perform properly Fe_{NO} and/or spirometry measurements and Fe_{NO} outliers values.

2.3.2. Group II (healthy-validation-group)

Seventeen additional healthy subjects (four females) older 50 years and more, visiting the Functional Exploration Laboratory from October to December 2014 were included. They met the inclusion criteria of the present study but did not participate in the first part.

2.3.3. Group III (asthmatic-validation-group)

Ten (nine females) known asthmatic patients older 50 years and more addressed for lung function testing were included. Asthma diagnosis was established by a pulmonologist based on medical history, respiratory symptoms and a positive bronchodilator response (increase in *forced-vital-capacity* (FVC) and/or *1st second-forced-expiratory-volume* (FEV_1) >12% initial and 200 mL after inhalation of a short-acting β_2-agonist). Enrolled asthmatic elderly patients were clinically stable and not currently (within three weeks before Fe_{NO} measurements) receiving corticosteroids.

2.4. Medical questionnaire

A medical questionnaire [25] was used to assess numerous subject characteristics.

Age (years) was taken as the number of complete years from birth to the date of the measurements. Height (cm) and weight (kg) were measured using a height gage with shoes removed, heels coupled, back straight and subject without heavy clothes. *Body-mass-index* was calculated ($BMI = weight/height^2$). Two groups of