

Original article

Passive smoking is a major determinant of exhaled nitric oxide levels in allergic asthmatic children

Background: Fraction of exhaled nitric oxide (FeNO) is considered, by some authors, to be a treatment follow-up parameter in allergic asthmatics. However, factors such as active smoking can influence NO production and must be taken into account in the interpretation of FeNO values. In children, the evidence in favour of an impact of passive smoking (PS) on FeNO values is controversial. The aim of this study was to evaluate the impact of chronic PS on FeNO in allergic asthmatic children.

Methods: Seventy nontreated allergic asthmatic children over 5 years of age, exposed and unexposed to PS, underwent measurement of FeNO, spirometry, and allergic tests (skin prick tests, total and specific serum IgE, and blood eosinophilia). Children were considered to be exposed to PS when at least 1 cigarette per day was declared to be smoked at home.

Results: Geometric mean FeNO value in 22 children exposed to PS was 26.3 ± 1.5 ppb vs 56.3 ± 1.7 ppb in 48 children unexposed ($P < 0.001$). After adjustment for age, blood eosinophilia, allergic sensitizations, total IgE, dust mite sensitization and asthma severity, multivariate analysis showed that PS exposure was negatively associated with FeNO values ($P = 0.0001$) and was the primary determinant of FeNO variations.

Conclusion: Passive smoking lowers FeNO, and might be a major determinant of FeNO levels in nontreated allergic asthmatic children.

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It is now suggested that measurement of Fraction of exhaled NO (FeNO) reflects bronchial inflammation in allergic asthmatic subjects (1–3). Some authors propose this measure as a valuable noninvasive method for the therapeutic follow-up of these patients (3–5). Some factors such as exposure to tobacco smoke can, however, interfere with the results of FeNO measurement. The effect of smoking on FeNO values has been investigated in many studies, which provide consistent evidence that daily active smoking significantly lead to a persistent decrease in FeNO levels in both healthy (6–13) and asthmatic adults (7, 14). However, less than 1 month after cessation of active smoking, FeNO values become similar to those of controls (11, 12). Furthermore, acute cigarette smoke exposure produces a rapid and even more marked decrease in FeNO values (13). However, this reduction is

transient, with a rapid return to baseline values only about 15 min following exposure. The effect of acute exposure to passive smoke (PS) has also been studied in adults (6, 15). Experimentally, in healthy nonsmoking subjects, PS exposure induces a significant reduction in FeNO levels during a few minutes postexposure. This reduction is transitory (6, 15). In healthy children, no study has yet demonstrated a link between PS and FeNO values (2, 16–19) whereas, in asthmatic children, the published results are discordant. Two reports have described no effect of PS on FeNO values (2, 19), a third provided evidence for a negative feedback effect (17), while the latest study showed that exposure to PS was associated with increased FeNO values in young children (20). These discrepancies are most probably due to methodological bias: small sample sizes, heterogeneous study populations and lack of control for potential confounding factors.

In this context, the objective of our study was to evaluate using multivariate analysis, the impact of daily PS exposure on variations in FeNO values in a clinically well-characterized and homogeneous population of asthmatic allergic children.

Abbreviations: ATS, American Thoracic Society; ERS, European Respiratory Society; FeNO, fraction of exhaled NO; FEF_{25–75}, forced expiratory flow between 25–75% of forced vital capacity; FEV₁, forced expiratory volume in 1 s; IgE, immunoglobulin E; iNOS, inducible NO synthase; NO, nitric oxide; PS, passive smoking; PV, predicted value; SPT, skin prick test.

Materials and methods

Study design and patients

This cross-sectional study compared children who were exposed and unexposed to PS. Children included in this study were selected among patients referred by their general practitioners for exploration of their asthma in the Asthma and Allergies Pediatric Centre of the Groupe Hospitalier Trousseau-La Roche Guyon, Assistance Publique-Hôpitaux de Paris.

Inclusion and exclusion criteria

We included children over the age of 5 years presenting nontreated mild to moderate persistent allergic asthma, all well controlled since at least 1 month before inclusion. Asthma was medically diagnosed, based on a history of recurrent wheezing and dyspnea attacks, with proven β_2 -agonist-reversible flow limitation. Asthma severity and the level of asthma control were evaluated according to the Global Initiative for Asthma (GINA 2006) guidelines.

In order to limit factors which can change FeNO values, we have not included nonallergic children and those receiving a current anti-inflammatory treatment [oral or inhaled or intranasal corticosteroids (21), anti-leukotriene (22)] or H1 receptor antagonists. Only short-acting β_2 agonist treatment was accepted because it not modifies FeNO values. Children with an asthma attack during the previous 2 weeks, symptomatic allergic rhinitis during the previous 2 weeks, or acute respiratory tract infection during the previous 4 weeks, were not included (23, 24). Children who were unable to perform the FeNO testing correctly and actively smoking children were also not included.

Approval was obtained from the French Ministry of Research Ethics and Scientific Committee and informed consent to participate was acquired from each participant and his parents. With respect to confidentiality of patient records, data handling for the study was authorised by the 'Commission Nationale d'Informatique et Libertés' (CNIL).

Methods

Measurement of FeNO. FeNO was measured by on-line method using a chemiluminescence NO analyser (NIOX[®]; Aerocrine AB, Solna, Sweden) before SPT and pulmonary function tests. The apparatus was regularly calibrated with a certified concentration of 213 ppb NO (Hoek Loos Specialty Gases, Amsterdam, the Netherlands) and delivered NO-free air that was inhaled by patients. The 'single expiratory breathing' technique was used at a fixed expiratory flow rate of 50 ml/s. At each session, three correctly executed exhalations were recorded and FeNO was measured according to ATS/ERS recommendations from 2005.

Lung function testing by spirometry. Each subject performed at least 3 physician-accepted and reproducible forced vital capacity curves using a SpiroDyn'R apparatus (Dyn'R, Muret, France) to measure Forced Expiratory Volume in 1 s (FEV₁) and Forced Expiratory Flow rate at 25–75% of forced vital capacity (FEF₂₅₋₇₅) before and 15 min after inhalation of salbutamol. Results were expressed as a percentage of published predicted values (% PV).

Determination of atopic status. Blood eosinophils were counted by an automat (Sysmex[®]; Roche Diagnostic, Meylan, France) and serum total IgE level expressed in kU/l, was determined by an immunonephelometric method (Pharmacia & Upjohn, Saint Quentin-en-Yvelines, France).

All children were screened by skin prick tests (SPT) for the following aeroallergens: house dust mite *Alternaria*, *Cladosporium*, *Aspergillus*, cat and dog danders, tree, grass, timothy grass, weed pollens, mugwort and *Blatta germanica* (Stallergènes SA, Antony, France). Allergic sensitization was defined by positive SPT (mean wheal diameter > 3 mm) and presence of specific IgE (CAP system; Pharmacia & Upjohn, Saint Quentin-en-Yvelines, France) greater than 0.35 kU/l. Polysensitized children were defined as presenting positive reaction to at least two different aeroallergens. Subjects with elevated total serum IgE and/or total blood eosinophil count but without serum specific IgE were excluded.

Evaluation of PS exposure. PS exposure was evaluated by a questionnaire that defined the variable 'number of cigarettes smoked at home per day' for each child. The exposed group (PS+) was comprised of children exposed to at least one cigarette per day, smoked at home, by the persons living together with the child.

Statistical analysis

The calculation of sample size was performed from the paediatric study of Barreto et al. (2). For α risk = 0.05 and power β = 0.80, the total number of subjects to include was 66.

Statistical analysis was performed on STATA RELEASE 8 software (Statcorp, College Station, TX, USA). Normal distribution was tested by the Shapiro-Wilks test and calculations were performed on normal or log-transformed variables. Inter-group patient characteristics were compared by variance analysis or chi-square test. A multiple linear regression model was then built to evaluate the impact of PS exposure on FeNO values after adjustment for confounders or risk factors of FeNO values ($P < 0.25$) such as age, blood eosinophilia, total serum IgE (continuous variables), asthma severity, allergic sensitization and dust mite sensitization (nominal variables). To complete the study, a stepwise procedure was performed. The threshold of significance was 0.05 for all tests.

Results

Subject characteristics

During the 6 months study, 119 children, 5 years or older, had been referred for exploration of asthma in stable period. Among those children, 70 controlled asthma allergic children suffering from mild to moderate stage of the disease were included in this study.

49 children were excluded for the following reasons: differential diagnosis of obstructive disease: 5; nonallergic asthma: 25; uncontrolled asthma: 6; acute respiratory tract infection: 3; current anti-inflammatory anti-asthmatic treatment: 4; impossibility to perform FeNO measurement: 5; and refusal to participate: 1.

Our study population was predominantly composed of boys (62%), with mean asthma duration of 4.8 years, and with mild persistent asthma in 71% of cases. Features of allergy were very marked, as reflected by the mean value of total IgE serum concentration greater than 600 kU/l, the mean blood eosinophil count close to 600/mm³, the high percentage of blood eosinophils (>8%) and the prevalence of polysensitization (49%).

On-line measure of FeNO was performed successfully on 70 children included in this study. No adverse effect was reported. The mean value of FeNO $\pm$ 1SD obtained in all included subjects was 52.2 ± 29.9 ppb. The mean number $\pm$ 1SD of cigarettes smoked at home per day was 9.4 ± 5.0 cigarettes for PS+ group. The two groups did not significantly differ for any of the other parameters related to demographics, asthma characteristics, allergic features and lung function (Table 1).

Factors associated with FeNO

After bivariate analysis, FeNO levels were significantly associated with asthma severity, dust mite sensitization. A significant, positive correlation was observed between FeNO values and age, height, weight and blood eosinophilia (Table 2). Conversely, PS exposure was negatively correlated with FeNO values. The FeNO values in exposed and unexposed children are shown in Fig. 1. The geometric mean FeNO value was significantly lower in the PS+ group (26.3 ± 1.5 ppb) than in the PS- group (56.3 ± 1.7) ($P < 0.001$). We found a significant negative correlation between FeNO values and number of cigarettes smoked at home ($r = -0.41$, $P < 0.001$) (Fig. 2). Other factors did not significantly associated: sex, asthma duration, number of sensitizations, total IgE serum concentration and lung function parameters.

The multivariate analysis confirmed these results. The multiple linear regression model including seven variables (age, blood eosinophilia, asthma severity, number of cigarettes smoked at home, total serum IgE level, allergic sensitizations and house dust mite sensitization), was highly significant ($P < 0.001$) and accounted for 64% of total FeNO variance (Table 3). In this model, five parameters remained significantly associated with FeNO levels, namely the number of cigarettes smoked at home, blood eosinophilia, age, asthma severity and dust mite sensitization.

The stepwise procedure revealed the large contribution of the 'number of cigarettes' variable to the variability of FeNO values, as it accounted alone for 33% of FeNO variance. The combination of two variables, number of cigarettes and blood eosinophilia, accounted for 45% of FeNO variance. When the number of cigarettes, eosinophilia, and age were added to the model, up to 55% of FeNO variance could be explained. Finally, all five variables accounted for 62.6% of FeNO variance.

Discussion

The present study is the first primarily designed to evaluate the effect of daily PS on FeNO measurement in asthmatic allergic children taking in account the variables that can influence FeNO values. We demonstrated a

Table 1. Characteristics of the two groups of children: unexposed and exposed to PS

Characteristics	PS- children (n = 48)	PS+ children (n = 22)	P-value
Demographics			
Age (years)*	9.5 ± 2.5	9.0 ± 3.0	0.47
Boys†	33 (68.7%)	11 (50.0%)	0.13
Weight (kg)*	34.1 ± 12.0	32.1 ± 12.3	0.52
Height (cm)*	136.4 ± 14.9	132.0 ± 16.2	0.28
Clinical parameters			
Asthma duration (years)*	5.1 ± 2.8	4.3 ± 3.5	0.29
Asthma severity			
Mild†	32 (66.6%)	18 (81.8%)	0.19
Moderate†	16 (33.3%)	4 (18.2%)	
Allergic features			
Total IgE (kU/l)*	658.9 ± 870.8 median = 397	629.3 ± 1032.7 median = 301	0.90
Monosensitized†	22 (45.8%)	14 (63.6%)	0.16
Polysensitized†	26 (54.2%)	8 (36.4%)	
Blood eosinophils count (cell/mm ³)*	590.2 ± 248.6 median = 551	623.9 ± 437.6 median = 545	0.68
% Blood eosinophils*	8.7 ± 3.6	8.3 ± 5.1	0.69
Lung function			
FEV ₁ (%PV)*	100.6 ± 13.8	106.0 ± 25.5	0.25
FEF ₂₅₋₇₅ (%PV)*	82.0 ± 24.1	80.5 ± 23.1	0.83

PS- children: children unexposed to PS; PS+ children: children exposed to PS; FEV₁: forced expiratory volume in 1 s (% of predicted values); FEF₂₅₋₇₅: forced expiratory flow between 25% and 75% of forced vital capacity (% of predicted values); 'Moderate persistent asthma' as defined in GINA 2006 guidelines; 'Mild persistent asthma' as defined in GINA 2006 guidelines; Monosensitized = children presenting positive reaction to one aeroallergen; Polysensitized = children presenting positive reaction to at least two different aeroallergens.

*Values are expressed as mean $\pm$ 1 SD.

†Values are expressed as absolute number and percentage of the corresponding population.

Table 2. Factors associated with FeNO values in bivariate analysis (linear regression)

Parameters	β (SE)	P-value	R^2 (%)
Age	0.069 (0.025)	0.007	10.2
Weight*	0.428 (0.201)	0.037	6.2
Height	0.012 (0.004)	0.007	10.1
Asthma duration	0.013 (0.023)	0.567	0.5
Total serum IgE*	0.115 (0.061)	0.064	5.0
% Eosinophilia	0.058 (0.016)	0.0001	16.7
FEV ₁ *	0.055 (0.058)	0.925	0.01
FEF ₂₅₋₇₅	0.003 (0.003)	0.364	1.4
Cigarette number*	-0.326 (0.056)	0.0001	33.0
Sex	-0.082 (0.144)	0.572	0.5
Asthma severity	0.221 (0.108)	0.045	5.8
Allergic sensitization	0.156 (0.131)	0.237	2.0
Dust mite sensitization	0.308 (0.150)	0.044	5.8

β (SE): regression coefficient and its standard error; R^2 : Coefficient of determination; FEV₁: forced expiratory volume in 1 s (% of predicted values); FEF₂₅₋₇₅: forced expiratory flow between 25% and 75% of forced vital capacity (% of predicted values).

*Variables log-transformed.

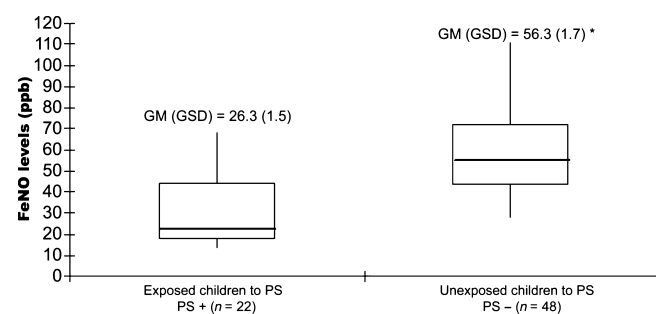


Figure 1. FeNO values in Passive Smoke exposed (PS+) ($n = 22$) and unexposed (PS-) ($n = 48$) allergic asthmatic children. Data are presented as geometric mean (GM), geometric standard deviation (GSD), 25th and 75th percentiles for boxes, 5th and 95th percentiles. * $P < 0.001$.

Table 3. Regression coefficients corresponding to change in FeNO in relation with cigarette number, unadjusted (first line) and adjusted (other lines) for other factors (stepwise multivariate regression)

Parameters	β (SE)	P-value	R^2 (%)
Cigarette number*	-0.33 (0.06)	0.0001	33.0
% Eosinophilia	-0.30 (0.05)	0.0001	45.2
Age	-0.30 (0.05)	0.0001	55.1
Asthma severity	-0.29 (0.06)	0.014	59.1
Dust mite sensitization	-0.28 (0.04)	0.017	62.6
Allergic sensitization	-0.28 (0.04)	0.13	63.9
Total serum IgE*	-0.28 (0.04)	0.27	64.2

β (SE): regression coefficient and its standard error; R^2 : coefficient of determination.

*Variables log-transformed.

clear, negative linear correlation between daily PS and FeNO values in allergic asthmatic children after adjustment for various confounders. The stepwise procedure

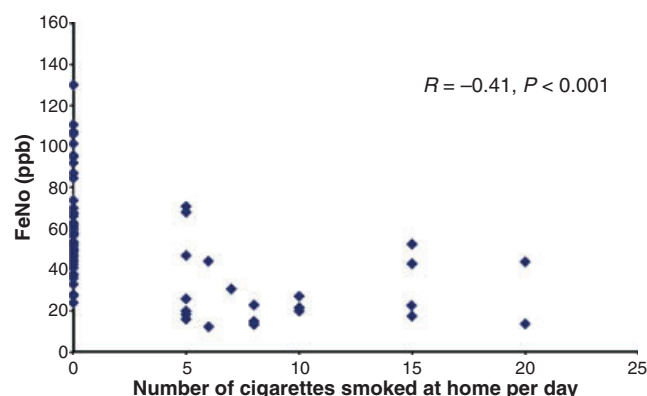


Figure 2. Negative correlation between FeNO values and the number of cigarettes smoked at home per day.

has shown that PS is the major determinant of FeNO values, followed by blood eosinophilia, age and asthma severity. PS alone accounted for 33% of FeNO variance.

The strict allergic phenotype in our population of asthmatics and the absence of current anti-inflammatory treatment, probably explain the high mean FeNO values observed in this study compared to certain values reported elsewhere (25).

The present work was specifically designed in order to minimise potential methodological bias by a strict selection of subjects and by clinical and biological validation of results. Firstly, asthma was diagnosed medically and not by a self-administered questionnaire, thus avoiding classification bias. Secondly, children fulfilling the principal conditions, in which FeNO values are known to be modified (acute asthma attack, allergic rhinitis or anti-inflammatory treatment), were excluded from the study. In addition, our study excluded nonallergic asthmatic children and the allergic features of the study sample were assessed through SPT and laboratory work-up (blood eosinophilia, total IgE serum level and specific IgE) since allergic status is known to be an important determinant factor in the increase of NO production (23, 24, 26). Finally, the present study was conducted in a homogeneous population and used multivariate analysis.

Exposure to PS was assessed on the basis of information reported by parents concerning their smoking habits. The advantage of this mode of data collection is that it can be easily obtained in routine practice, but it can induce a classification bias as the parents of the most severely affected children tend to underestimate their tobacco consumption at home.

This investigation aimed to study the impact of chronic exposure to PS. It was difficult for us to have an objective measure of such an exposure: urinary cotinine is affected by inter-individual variability in cotinine excretion levels for similar exposure, and a relatively short half life of 20 h (is a marker of recent exposure), and it was not

possible to us to perform measurements in hair samples. However, in clinical practice, clinicians using FeNO values will not check and adjust for a cotinine measure of exposure but rely on parental reporting, so this study can be regarded as pragmatic.

The correlation between FeNO values and age had already been reported by several authors (16, 23), though not by others (3). In contrast, no association between FeNO values and sex was observed in the present study, in line with other studies (2, 16). No significant association was found between the number of allergic sensitizations and FeNO values. Some studies have demonstrated an association between FeNO values and the number of positive SPT (16, 23). However, Silvestri et al. (26), did not confirm these results. Asthma severity, was on the other hand, clearly related to FeNO values, in line with the work of Delgado-Corcoran et al. (27) but not with the study by Griese et al. (28).

Few previous studies have evaluated the relation between FeNO values and PS exposure in asthmatic children (2, 17, 19). Our results are consistent with those obtained by Warke et al. (17), who found FeNO levels to be lower in exposed children as opposed to unexposed children. In contrast, the studies by Barreto et al. (2) and Dinakar et al. (19) failed to demonstrate a difference of FeNO levels in asthmatic children whether exposed or unexposed to PS, probably due to a lack of power (insufficient sample size) and to the heterogeneity of the studied population. For example, Barreto et al. (2) included only 41 asthmatic children and only 25 of them were atopic. In the study by Dinakar et al. (19), only 13 PS- and 11 PS+ asthmatic children were investigated and more than one half of the children were taking anti-asthmatic treatments, able to reduce FeNO production. Moreover, in these studies, the statistical conducted analyses were mostly bivariate, and thus could not take into account potential confounders or FeNO risk factors such as age, allergen sensitizations, inhaled corticosteroid treatment and asthma severity.

In our study, an effect of PS on FeNO values was demonstrated with a relatively smaller sample size compared to the study by Warke et al. (17), which included 174 asthmatic children. Moreover, they did not take in account confounding factors.

Franklin et al. (20) published an opposite result, but they investigated a different population (infants younger than 2 years of age) and their sample was heterogeneous. In effect, only 36 of the 78 children included, had a history of wheezing. However, it is established that at the infant, the majority of wheezers are not authentic asthmatics but transient wheezers. Furthermore, the atopic status of these children was not investigated. This result, conflicting with those of all the other studies, whether in adults or in children, could be explained by the relative important proportion of allergic asthma in the group of infants exposed to PS.

The results reported here are also in agreement with data concerning active smoking in both healthy and asthmatic adults (6–14). To the best of our knowledge, no epidemiological study, on the impact of PS, has been published in adults. Only two experimental works have demonstrated a real, but transient, reduction of FeNO values after brief and acute exposure to PS (6, 15). Our results also demonstrate the existence of a highly, significant negative correlation ($r = -0.41$, $P < 0.001$). This result is in agreement with that of Kharitonov et al. (13) which, showed a negative correlation between FeNO values and the number of cigarettes smoked per day in adult ($r = -0.77$, $P < 0.001$). No previous study has demonstrated such a relation in children. In the subgroup of exposed children we do not find a significant correlation between number of cigarettes smoked and FeNO, perhaps due to few exposed children and the weak contrast in the distribution of cigarettes smoked at home (range: 5–20).

This effect of PS on FeNO values was demonstrated in a selected population, so we think that in a more heterogeneous population (as encountered in real life), this effect would probably be lesser.

Two hypotheses have been put forward to explain the reduction of NO production in smokers. First of all, changes in local osmolarity in the airways due to cigarette smoke could reduce the NO concentration in expired air (29). Secondly, the existence of negative feedback on inducible NO synthase (iNOS) production could also account for this reduction (13, 30), since tobacco smoke contains high concentrations of NO (9, 31). This exogenous NO could both inhibit iNOS activity and down regulate its genetic expression (30, 32).

The mechanisms responsible for reduction of NO production related to PS may differ according to the type of exposure. Acute experimental exposure to PS induces, a marked, but transient reduction of NO production (6, 15), related to a modification in local osmolarity and negative feedback of iNOS activity. In contrast, the mechanism involved in the case of daily PS is not known. One hypothesis is that the progressive, negative feedback mechanism leading to sustained inhibition of iNOS gene expression (30, 32) which, could explain the lasting reduction of FeNO production observed in the context of daily exposure.

In conclusion, this study shows that PS significantly lowers FeNO values, after adjustment for various confounders and risk factors, and might be a major determinant of FeNO levels in untreated allergic asthmatic children exposed to daily PS. It, therefore, appears essential to take this exposure into account when interpreting results of FeNO measurements in this category of children, both in clinical practice and in research approach. Further studies are required to determine appropriate management of the effect of PS on FeNO values and to evaluate this effect on treated asthmatic children.

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