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Research article

Correlation between fractional exhaled nitric oxide level and clinical outcomes among childhood asthmatic patients: community hospital-based perspective

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ABSTRACT

A fractional exhaled nitric oxide (FeNO) device has been developed for the evaluation of clinical control in asthmatic patients, instead of for uses in only analyzing clinical data and spirometry. The implementation of the FeNO device in daily practice has demonstrated both positive and negative results. Community hospital settings in Thailand have never used this method for the evaluation of disease control in asthmatics. The aim of this study was to assess the correlation between FeNO levels and asthma control, as determined by physicians. We recruited asthmatic patients aged from 4-15-years-old (after informed consent approval was obtained from their guardians) from May 15, 2018, to July 20, 2019. All of the patients had already been diagnosed as having asthma by physicians and had been prescribed inhaled corticosteroid medications. After routine visits, skin prick tests with 8 aeroallergens were performed. If a positive result was shown for at least 1 allergen, then the FeNO device was applied for the assessment of the level of inflammation. Data were analyzed by using SPSS Statistics version 21.0. Agreement index and Kruskal Wallis tests were used to measure the correlations. From 178 asthmatic patients, the mean age was 94.9 ± 36.75 months, and 59% of them were male. The educational levels of the guardians of the patients mostly consisted of primary school, and the household income was less than 333 US dollars per month. Inhaled corticosteroids were prescribed among the patients for disease control. The correlation between the FeNO level and the control level demonstrated a high agreement (accuracy index: 91.57%). The medians of the mean wheal diameters of *Dermatophagoids pteronyssinus*, *Dermatophagoids farinae* and *Cladosporium* spp. were significantly related to the FeNO level (with p-values of 0.024, 0.003 and 0.045, respectively). Conversely, a number of positive skin responses to aeroallergens were not related with the FeNO level. In conclusion, a lower level of FeNO correlates with good asthma control level in pediatric allergic asthma. The medians of the mean wheal diameters of *Dermatophagoids pteronyssinus*, *Dermatophagoids farinae* and *Cladosporium* spp. correlated with higher FeNO levels. A device assessing FeNO seems beneficial for evaluating the level of disease control among childhood asthmatic patients in a primary care setting.

1. Introduction

Childhood asthma has been a significant public health chronic disease for decades [1]. Data from ISAAC III (both from developed and developing countries) showed that the prevalence of childhood asthma among patients aged 6–7 years and 13–14 years were 11.7% and 14.1%, respectively [2]. A study among primary school children in Bangkok, Thailand, showed a prevalence of asthma of 9% [3]. Data collected from primary school children in Chiang Mai, Northern Thailand, revealed that the prevalence of asthma changed from 5.5% in 1995 to 7.8% in 2001 [4]. A prospective cohort study that was conducted for 30 years among childhood asthmatics in the Netherlands showed that 50% of initial registered patients had been followed up [5]. There are various dimensions of negative impacts from this condition, such as complications from the disease or from the treatment (especially in regard to inhaled corticosteroids) that affected height rates [6], impaired quality of life and caused school absences, work loss, costs of treatment or other cost losses of opportunities [7].

Challenges have been uncorrected for childhood asthma managements, such as the timely and definitive diagnosis (especially in patients

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under the age of 5 years-old), which usually relied on risk factors and clinical data. An objective measurement via spirometry was also difficult to implement among this age group [8]. In addition, the appropriate and continuous use of inhaled corticosteroids has been a serious obstacle [9]. Regular follow-ups, prompt initial management (in cases of acute asthmatic exacerbations) [10] and environmental controls for allergen avoidance remain to be seriously considered [11].

A standard asthma management guideline has been implemented in a community hospital in Thailand by establishing the Easy Asthma Clinic [12]. Multidisciplinary health-related personnel, including physicians, pharmacists, nurses and physical therapists, have cordially planned and run the program [12]. An evaluation comparing pre- and post-program implementations showed considerable outcomes. Nevertheless, due to the chronic nature of the disease and the complexities of allergen avoidance methods, the control of childhood asthma and adverse clinical outcomes (acute exacerbation, emergency visits and hospital admissions) have remained a considerable challenge for health care providers [13].

Most of the affected patients with asthma have usually exhibited their symptoms since early childhood [14]. Genetic and environmental factors play key roles for disease progression. Among childhood asthma, the allergic and eosinophilic types are more common. After allergens are inhaled through the airway passage, intracellular changes occur, especially in the bronchial trees. Inflammation in various levels of the airway tubes will gradually induce the release of mediators, such as nitric oxide, carbon monoxide and breath condensates, among other mediators. Repeated inflammation and chronicity can induce the thickness of the bronchial reticular basement membrane and fibrosis in the sub-basement. In addition, the smooth muscles of the bronchial tree can become hyperplasic and hypertrophic with increased angiogenesis [15]. Nitric oxide is a key biological marker in asthma, and it is produced by inducible nitric oxide synthase [16, 17]. This indicates a degree of steroid responsiveness. In cases of sustained high levels of exhaled nitric oxide and continued uses of inhaled steroids, possible causes may be due to medical non-adherence, inappropriate medical techniques or failures of complete allergen avoidance [18].

Fractional exhaled nitric oxide (FeNO) has been implemented for decades in daily clinical practices in order to measure respiratory tract inflammation. This technique was performed according to the eosinophilic type of asthmatic bronchial pathology, which is highly responsive to inhaled corticosteroid medication. Moreover, FeNO has also been used for clinical monitoring or dose-adjudged medication, rather than relying solely on histories, physical examinations and spirometry [19, 20].

At the level of a community hospital in Thailand, the FeNO procedure has not yet been implemented for asthmatic patients. We hypothesized that pediatric asthmatic patients who were classified as controlled or partially (not well) controlled would have lower levels of FeNO. We also hoped that the results and implications of this study would be beneficial for patients in daily practice, especially for adjustments of medications. In addition, clinical researchers may pay more attention to the use of this device that yields clear objective measurements, rather than machines that only rely on clinical data. This study aimed to evaluate the correlation between inflammation levels in the exhaled air of childhood asthmatics by using FeNO levels, compared to the use of conventional asthmatic assessments by physicians and health related personnel. Additionally, we aimed to determine whether FeNO levels in the exhaled air of childhood asthmatics were associated with the number of positive skin prick tests or median mean wheals from this procedure.

2. Materials and methods

2.1. Study design and sample size

This study performed a cross-sectional descriptive analysis of childhood eosinophilic asthma, which was sensitized to at least 1 aeroallergen. FeNO was used to measure the inflammatory levels of the exhaled air of patients at the out-patient department of the Thasala hospital, Nakhon Si Thammarat, Southern Thailand. We recruited eligible participants who were aged 4–15 years from May 15, 2018 to July 20, 2019. The sample size of the study was computed based on the following formula: $n = (Z_{\alpha/2})^2 PQ/d^2$ [21], in which n = the initial number of patients for calculating the sample size, $Z_{\alpha/2}$ = the standard normal distribution (1.96), P = the sensitivity of the FeNO device (0.8) [22], Q = (1 – P = 0.2), d (acceptable error) = 0.1 and n = 61.47. Additionally, in 2017, the data from the Thasala hospital, Nakhon Si Thammarat showed that if the prevalence of uncontrolled childhood asthma was 35%, then the total number of asthmatic patients needed at be at least 61.47/0.35 = 175.63 (176). There were a total of 230 childhood asthmatic patients who were registered in this hospital. We used purposive sampling via consecutive enrollment if the individuals met the inclusion criteria. In total, we enrolled 178 childhood asthmatic participants. This study was approved by the Institutional Review Board (IRB) of Walailak University (Approval Number WUEC-16-144-01). Written informed consent was obtained from all of the participants before they enrolled in this study.

2.2. Eligibility criteria

The inclusion criteria consisted of the following: children who were aged 4–15 years, children with physician-diagnosed asthma, children who were registered at the out-patient department in the Thasala hospital, Nakhon Si Thammarat, Southern Thailand, children who were treated with inhaled corticosteroids for at least 6 months, children with an avoidance from antihistamine or related groups for at least 7 days, children who tested positive to at least 1 aeroallergen on a skin prick test cases in which the patient and guardian complied with this study. The exclusion criteria included patients who had co-morbidities, such as heart disease, chronic lung disease, neurological disease or psychiatric disease, patients who had recent respiratory infections within 4 weeks prior to enrollment in the study, patients who avoided the performance of skin prick tests or exhaled nitric oxide tests.

2.3. Questionnaire

Demographic data, history, clinical symptoms and managements were included in this questionnaire. Research assistants directly performed an interview with the guardian and the patient. In addition, we also utilized clinical judgment from a pediatrician regarding disease control status by using an asthma control test. This tool was comprised of a 5-item self-administered questionnaire that included the following factors: the frequency of asthmatic symptoms, daily activity limitations, sleep interference, the use of rescue medication and overall self-ratings of asthma control in the past 4 weeks. Numerical score ranges from 5-25 indicated the following: controlled (>19 points), not well controlled (16–19 points) or very poorly controlled (<16 points) [23].

2.4. Skin prick test

Skin prick testing with eight common indoor aeroallergens was performed and included *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Periplaneta americana*, cat dander, dog dander, Para grass (*Brachiaria mutica*), Careless weed (*Amaranthus retroflexus*) and *Cladosporium spp*. All of the standard allergen extract panels were obtained from Greater Pharma, Nakhon Phathom, Thailand. Positive and negative controls, including histamine and glycerinated phenol-saline, respectively, were used. Skin prick testing was applied on the volar surface of the forearm, and the skin reaction was then evaluated 15 min later. Positive test results were defined as a reaction of redness and wheal (per Dogru et al. and Hosseini et al.) [24, 25]. Individuals who had positive test results for at least one aeroallergen were defined as having an allergen sensitization and were further processed for data analysis.

Table 1. Demographic characteristics of the participants (n = 178).

Demographic characteristics	Mean ± SD or n (%)
Age	94.9 ± 36.75 months
Sex	
Male	105 (59%)
Female	73 (41%)
Body Mass Index (Kg/m^2)	16.99 ± 3.62
Religion	
Buddhism	109 (61.2%)
Muslim	69 (38.8%)
Race	
Thai	177 (99.4%)
Other	1 (0.6%)
Sibling's order in family	
First order	88 (49.4%)
Second order	50 (28.1%)
Third order and more	40 (22.5%)
Total siblings in family	
One	49 (27.5%)
Two	71 (39.9%)
≥Three	58 (32.6%)
Family monthly incomes	
Less than 333 US dollars	74 (41.6%)
333-600 US dollars	64 (36%)
601-1,000 US dollars	28 (5.7%)
Greater than 1,000 US dollars	12 (6.7%)
Guardian	
Mother	124 (69.7%)
Father	10 (5.6%)
Relative	44 (24.7%)
Guardian's educational level	
Primary school or less	80 (44.9%)
Secondary school	69 (38.8%)
Vocation or diploma	12 (6.7%)
Bachelor's degree or more	17 (9.6%)

2.5. Exhaled nitric oxide measurement device

Exhaled nitric oxide measurements were conducted by using a device from Bedfont, UK, using the electrochemical sensor principle. Participants had to stand in the upright position and were then told to hold the equipment with both hands. After complete exhalations, the participants had to deeply inspire via a mouth piece with a nitric oxide filtration membrane and continuously blew out air for 10 s. All of these actions had to be performed 3 times, and only the best value was used for analysis. The procedure was performed according to the manufacturer's instructions and American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations. Exhaled nitric oxide levels were classified as follows: a low level at less than 20 ppb; a normal level at 20–35 ppb; and a high level at greater than 35 ppb [26, 27].

2.6. Statistical analyses

Descriptive statistical analyses were used in this study to demonstrate demographic, clinical and laboratory data. Means, standard deviations (SD) and ranges were used for the numerical variables. Proportions and percentages were used for the categorical variables. A correlation between the exhaled nitric oxide level and the asthma control level was conducted by using agreement and kappa indices. For the correlation between the exhaled nitric oxide level and the median of the mean wheal diameter of the aeroallergen skin prick test, the Kruskal Wallis Test and the Pearson Correlation (r) were used. All of the statistical analyses of the demographic characteristics, clinical characteristics and laboratory data were performed by using PSPP, version 1.2.0 (2016 Free Software Foundation, Inc.). A p-value of <0.05 was considered to be statistically significant.

3. Results

3.1. Demographic characteristics of the participants

A total number of 178 participants were included from May 15, 2018 to July 20, 2019, with a mean age 94.9 ± 36.75 months. The youngest patient was 48-months-old, whereas the oldest patient was 180-months-old. Males were the predominant sex (105 participants, 59%). The mean body mass index was 16.99 ± 3.62 kg/m^2. Most of the patients were Thai (177 participants, 99%), and the majority of patients were Buddhist (109 participants, 61.2%), while the rest of the patients were Muslim. Most of the patients had guardians who were their mother (124 participants, 69.7 %). Most of the guardians had graduated at the primary school level (80 participants, 44.9%). The monthly income of the family was less than 333 US dollars (74 participants, 41.6 %), as is shown in Table 1.

3.2. Clinical characteristics of the participants

Comorbidities were demonstrated to be allergic rhinitis, food allergy, atopic dermatitis, allergic conjunctivitis, anaphylaxis and other factors (38.2%, 11.8%, 11.2%, 4.5%, 3.9% and 9.6%, respectively). Sixty-six participants (37.1%) showed the onset of first wheezing, and the physician diagnosed them as having asthma in the first year of life. Seventy-one participants (39.9%) had at least one family member living with an allergic condition. Seventy-six participants (42.7%) used at least one dose of a bronchodilator in the past 6 months, as shown in Table 2.

3.3. Skin prick test results with aeroallergens

Common aeroallergens were used in this study, such as *Dermatophagoids pteronyssinus*, *Dermatophagoids farinae*, cockroach, cat dander, dog dander, Para grass (*Brachiaria mutica*), Careless weed (*Amaranthus retroflexus*) and *Cladosporium* spp. Individuals with positive tests to at least

Table 2. Clinical characteristics of the participants (n = 178).

Clinical characteristics	Mean ± SD or n (%)
Comorbidities	
Allergic rhinitis	68 (38.2%)
Food allergy	21 (11.8%)
Atopic dermatitis	20 (11.2%)
Allergic conjunctivitis	8 (4.5%)
Anaphylaxis	7 (3.9%)
Others (such as drug allergy, insect sting, etc.)	17 (9.6%)
Onset of first wheezing and physician-diagnosed asthma	
Birth–3 years	134 (75.3%)
3–6 years	23 (12.9%)
6 years and greater	21 (11.8%)
Family history of allergy	71 (39.9%)
Father	22 (12.4%)
Mother	40 (22.5%)
Older brother/sister	18 (10.1%)
Younger brother/sister	11 (6.2%)
Medications used in the past 6 months	
Inhaled corticosteroid	145 (81.5%)
Inhaled bronchodilator	76 (42.7%)
Long-acting inhaled corticosteroid with bronchodilator	7 (3.9%)
Oral antihistamine	45 (25.3%)
Oral corticosteroid	1 (0.6%)

Table 3. Skin prick test results with aeroallergens (n = 178).

Aeroallergens	Positive Skin Prick Test n (%)
Dermatophagoids pteronyssinus	152 (85.4%)
Dermatophagoids farinae	137 (77.0%)
Cockroach	82 (46.1%)
Cat dander	42 (23.6%)
Dog dander	32 (18.0%)
Para grass (*Brachiaria mutica*)	23 (12.9%)
Careless weed (*Amaranthus retroflexus*)	22 (12.4%)
Cladosporium spp.	18 (10.1%)

Table 4. Correlation between the FeNO level and the asthma control level.

Asthma control level	FeNO level	
	0–35 ppb (normal)	>35 ppb (abnormal)
Controlled and partially controlled	161	12
Very poorly controlled	3	2

Agreement = 91.57%; kappa = 0.1764, p = 0.0034.

current states of inflammation and uncontrollable disease. In this study, the asthma control status level was defined by using patient histories, asthma control test scores and physical examinations from physicians. This study showed a high agreement between the FeNO level and the asthma control level, (with agreement of 91.57%, kappa = 0.1764 and p = 0.0034), as shown in Table 4.

3.5. Correlation between skin prick test results and the FeNO level

In childhood asthmatics, the FeNO level was categorized as being low if the level was less than 20 ppb; was normal if the level was 20–35 ppb; and was high if the level was greater than 35 ppb. When comparing the FeNO level with the medians of the mean wheal diameter of the 8 aeroallergens, the data showed correlations in *Dermatophagoids pteronyssinus*, *Dermatophagoids farinae* and *Cladosporium* spp., with p-values of 0.024, 0.003 and 0.045, respectively, as shown in Table 5.

3.6. Correlation between the FeNO level and the quantity of the positive skin prick test

From the 8 aeroallergens that were introduced via the intradermal route of all of the participants, there was no correlation between the

Table 5. Correlation between the medians of the mean wheal diameters and FeNO levels.

Aeroallergens	FeNO			p-value
	Less than 20 ppb	20–35 ppb	Greater than 35 ppb	
	Median (range)	Median (range)	Median (range)	
Dermatophagoids pteronyssinus	3 (0–15)	3 (0–15)	4.25 (0–22.5)	0.024*
Dermatophagoids farinae	3 (0–20)	3 (0–25)	4.25 (3–20)	0.003*
Cockroach	0 (0–10)	0 (0–5.5)	1.5 (0–12)	0.512
Cat dander	0 (0–4)	0 (0–9)	0 (0–3)	0.519
Dog dander	0 (0–3)	0 (0–4)	0 (0–0)	0.091
Para grass (*Brachiaria mutica*)	0 (0–3)	0 (0–3)	0 (0–0)	0.202
Cladosporium spp.	0 (0–3)	0 (0–0)	0 (0–3)	0.045*
Careless weed (*Amaranthus retroflexus*)	0 (0–3)	0 (0–3)	0 (0–0)	0.335

Median: the median of the mean wheal diameter by using the skin prick test.
* p-value < 0.05 indicates statistical significance.

Table 6. Correlation between the FeNO level and the quantity of the positive skin prick tests.

Quantity of aeroallergens with positive response	FeNO level		
	0–19 ppb	20–35 ppb	>35 ppb
1 aeroallergen	19	7	0
2-3 aeroallergens	81	20	12
≥3 aeroallergens	30	7	2

Agreement = 23.03%, Kappa = 0.0197, p > 0.05.

one aeroallergen were further analyzed. From 178 participants, *Dermatophagoids pteronyssinus* exhibited the most prevalence for positive skin prick tests (152 participants, 85.4%). *Dermatophagoids farinae*, cockroach, cat dander, dog dander, Para grass (*Brachiaria mutica*), Careless weed (*Amaranthus retroflexus*) and *Cladosporium* spp. exhibited the following positive test rates (137, 77%; 82, 46.1%; 42, 23.6%; 32, 18%; 23, 12.9%; 22, 12.4%; and 18, 10.1%, respectively), as shown in Table 3.

3.4. Correlation between the FeNO level and the asthma control status level

In allergic asthma, the FeNO level is the key indicator representing bronchial inflammation. A FeNO level of 35 ppb or greater is defined as being abnormally high in childhood asthmatics. This level is related with

FeNO level and the quantity of the positive skin prick tests, as shown in Table 6.

4. Discussion

Nitric oxide is a primary biological marker in eosinophilic asthma, which indicates steroid responsiveness. Recently, there have been numerous medical devices used for assessing exhaled nitric oxide, in order to evaluate asthma control after the implementation of inhaled corticosteroid uses in both adult and childhood asthmatics. The present study aimed to assess the correlation between inflammation levels in childhood asthmatic exhaled air by using the FeNO level, as well as to compare the use of the FeNO device with conventional methods, as

assessed by physicians and team members. In addition, we aimed to clarify whether FeNO levels in the exhaled air of childhood asthmatics were associated with the number of positive skin prick test responses.

To the best of our knowledge, this was the first study performed in primary care setting in Thailand using the FeNO device for assessing asthma control among childhood asthmatics. The study demonstrated a high correlation between FeNO levels in childhood asthmatics with asthma control levels (accuracy index: 91.57%). The normal or lower levels of FeNO (especially levels lower than 35 ppb) indicated better asthmatic control statuses that the participants showed. This was similar to reports from many previous studies [28, 29]. Nevertheless, a number of studies have shown conflicting results [30, 31, 32]. From the present study, 12 of the 178 participants were reported as having well controlled or not well (partially) controlled asthma; however, FeNO levels were shown to be higher than 35 ppb. This can be explained by the fact that the participants may have lacked appropriate technical knowledge for medication usage, a non-adherence for medication or unavoidable contact with allergens [18]. Furthermore, 3 participants were classified as having very poorly controlled asthma, but the FeNO levels were at a normal limit (less than 35 ppb). This may have resulted from unclear or incomplete information provided by the participants and guardians, which may have resulted in misclassified levels of disease control.

Skin prick tests with eight aeroallergens were performed in 178 participants, and positive tests were shown in individuals for at least 1 aeroallergen. *Dermatophagoids pteronyssinus* was the most prevalent positive response among childhood asthmatics in this study. In addition, *Dermatophagoids farinae*, cockroach, cat dander, dog dander, Para grass (*Brachiaria mutica*), Careless weed (*Amaranthus retroflexus*) and *Cladosporium* spp. also exhibited positive responses. This appeared to be similar to a study conducted in Thai childhood asthmatics [33]. The present study showed a correlation between a high level of FeNO and the medians of the mean wheal diameters of *Dermatophagoids pteronyssinus*, *Dermatophagoids farinae* and Cladosporium spp. This was in agreement with a study in Malaysian secondary schoolers (aged 13–14 years), which showed a high level of FeNO (greater than 20 ppb) in individuals who had positive tests for *Dermatophagoids pteronyssinus*, *Dermatophagoids farina* and cat dander [34]. For the quantity of positive tests to aeroallergens, we found no correlation with the FeNO level. This was in contrast with a study from China, which showed that poly-sensitization to aeroallergens had higher levels of FeNO than mono-sensitization among childhood asthmatics [35]. This difference may be attributed to the allergen exposure level, especially for indoor allergens like house dust mites. Barreto M et al. found that living with high levels of indoor house dust mites results in high levels of FeNO [36]. The participants in this study may live with different levels of allergen exposure; thus, they did not show correlations with FeNO levels.

The present study had some limitations, including lacks of objective measurements for eosinophilic parameters, medical adherence at home and quantification of allergen exposure. Future research should focus on these factors, in order to demonstrate more precise results of these associations. In addition, a cost-effectiveness analysis should be performed before the implementation of this device to daily practice in a primary care setting in developing countries.

5. Conclusion

This study demonstrated that a lower level of FeNO correlates with well controlled or partially controlled levels in pediatric allergic asthma. The medians of the mean wheal diameters of *Dermatophagoids pteronyssinus*, *Dermatophagoids farinae* and *Cladosporium* spp. correlated with a higher FeNO level. We believe that the FeNO device appears to be beneficial for assessing the level of disease control among childhood asthmatics. Future studies should focus on an objective measurements and a cost-effectiveness analysis before implementing the use of this device to daily practice in primary care settings.

Declarations

Author contribution statement

Theerapan Songnuy: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Prachyapan Petchuay: Performed the experiments; Wrote the paper.

Wongwat Jutiyon and Awirut Nurak: Performed the experiments.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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