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Cotinine Level Is Associated with Asthma Severity in Passive Smoker Children

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ABSTRACT

Asthma environmental triggers play important roles in severity of disease. Passive smoking could exacerbate asthma symptoms and enhance the decrease in lung function. Cotinine levels could be a reflection of passive exposure to the cigarette both in adults and pediatrics. The aim of this study was to determine degree of association of asthma severity and cotinine level as a marker of passive smoking.

In a cross-sectional study, 100 pediatric patients (under 10 years old) with asthma were enrolled, 50 of whom, had been exposed to passive smoking and 50 others included as controls. A complete clinical history, lab exam, and spirometry were performed. A sample of urine, serum and saliva was collected from all attendant patients and controls in the study after confirmation of diagnosis and determination of severity of asthma.

The results revealed that age, sex, age of onset of asthma, family history and allergic history were not significantly different between two groups of patients. According to GINA classification, percentage of patients with severe asthma was significantly higher in passive smoker group (p=0.001). Cotinine was significantly higher in passive smoker group compared to control group in serum (p=001), saliva (p=0.001), and urine (p=0.0014). In passive smoker group, cotinine levels were significantly higher in serum (p=0.001), urine (p=0.007), and saliva (p=0.01) of patients with severe asthma than moderate and mild asthma. Serum cotinine (OR: 1.81, 95% CI: 1.35-2.32, p=0.024), urine cotinine (OR: 3.56,95% CI = 1.29-5.53, p=0.01) and saliva cotinine (OR: 1.66, 95% CI: 1.23-1.98, p=0.031) were also significantly associated with higher risk of severe asthma.

Cotinine levels were higher in passive smokers compared to non-passive smokers. Besides, cotinine was a predictive risk factor for severe asthma.

Keywords: Asthma; Child; Cotinine

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INTRODUCTION

Asthma, as a chronic inflammatory disorder, is mainly associated with bronchial hyperreactivity (BHR). Asthmatic patients manifest elevated level of inflammatory responses and BHR in response to many common environmental allergens and triggers. Asthma environmental triggers and emotional factors[1,2,3] play important roles in severity of disease. Although patients vary tremendously in their response to different triggers, smoking is one of the widespread triggers of asthma.[4]It is believed that increased prevalence of asthma in adult during childhood is associated with environmental tobacco smoke exposure.[5]

There are more than billion cigarette smokers all around the world. According to World Health Organization (WHO) report, more than 700 million children are vulnerable to be passive smokers. Children with smoker parents are often exposed to high levels of environmental tobacco smoke, and children with asthma are particularly susceptible to the detrimental effects of passive smoking.[6]Passive smoking could exacerbate asthma symptoms and enhance the decrease in lung function.[7]Control of passive smoking is one of the ways to prevent and manage pediatric asthma.[8]

Serum cotinine levels could be a reflection of passive exposure to the cigarette both in adults and pediatrics.[9,10]Data indicate a 30minute exposure to smoke increases urinary cotinine and produces prominent airflow obstruction in human subjects particularly in asthmatics.[11]Environmental tobacco smoke exposure in school children have been correlated with urine cotinine measures. Cotinine is a metabolite and biomarker of nicotine which could provide valuable information regarding duration of passive exposure to cigarette smoke.[12]

Children with asthma are vulnerable to passive smokers' effects, but the degree of these effects are still obscure to many clinicians. Besides, association of passive smoking and asthma severity is still to be described. Results from human studies vary, reasonably explained the complexity of the experiment, which causes several variables to change at the same time.

In this study, we embarked on determining degree of association of asthma severity and cotinine level as a marker of passive smoker.

MATERIALS AND METHODS

The study was reviewed and approved by the University Review Board and hospital ethics committee and has been performed in accordance with the ethical standards laid down in an appropriate version of the 2000 Declaration of Helsinki. Information about trial was given comprehensively both orally and in written form to the parents. All parents gave their written informed consents prior to their inclusion in the study according to University Hospital Ethics Board Committee.

Patient Selection and Study Design

In a cross-sectional study, 100 patients with asthma were enrolled, 50 of whom, had been exposed to passive smoking and 50 others served as controls.

Inclusion criteria was pediatric patients less than 10 years old with asthma or BHR. Exclusion criteria were patients with upper or lower respiratory tract infections, any genetic or hereditary lung diseases such as cystic fibrosis or bronchiectasia.

Data Collection and Outcomes

Data were collected from parents who smoke and from their asthmatic children. Patients'demographic characteristics were recorded at the first visit. A complete clinical history,laboratery examinations,and spirometry were also performed. Thereafter, follow-up visits were performed 2 monthslater. A questionnaire was filled by parents referred to asthma clinic.Asthma severity was determined according to GINA guidelines.[13]

A sample of urine, serum and saliva was collected from all attendants in the study after confirmation of diagnosis and determination of severity of asthma. The samples were carried on ice and then transferred to the -80ºC fridge until cotinine measurement. Urine, serum and saliva cotinine were measured using an ELISA kit (according to manufacturer protocols).

Spirometry

Spirometry was performed in patients older than 5 years of age based on the standard module by measuring airflow limitation. However, pediatrics spirometry is a difficult task in many cases in ages between 4-6 years and under.

Statistical Analysis

Statistical calculations were conducted using SPSS 18 (Chicago, IL, USA). The parametric variables were presented as mean ±SD and were analyzed by student t-test or ANOVA and Pearson correlation test as appropriate. Statistical analysis was performed using Chi-Square or Mann-Whitney U-test and Spearman correlation coefficients for non-parametric samples. Multiple logistic regression was used to assess the association of cotinine level and asthma severity. $P<0.05$ was considered as statistically significant. Sample size was estimated using sample size calculator software with 95% confidence interval and $p<0.05$.

RESULTS

Total numbers of 100 patients were enrolled in the study,50 of whom had been passive smokers and 50 were not (control group). Three patients in control group and 2 patients in passive smoker group were lost in follow up. Age of diagnosis at our clinic was not significantly different between the two groups of patients (Table1). Age of onset of disease in passive smoker group was 4.27 ± 1.92 years and5.12 ± 3.6 years for control group ($p=0.34$). Mann Whitney U test showed that the birth order was not significantly different between the two groups ($p=0.26$). Our results did not show significant differences between the passive smokers and control group in case of positive history for low birth weight ($p=0.08$), and caesarean section ($p=0.15$). Total years of exposure to cigarette were 5.2±3.56 years in passive smokers. Moreover, gender distribution was not different between those in passive smokers and control groups. Smokers were one of the parents who were mostly father and only in 4 cases, it was the mother. Family history of asthma in parents and other siblings is depicted in Table2 which was not significantly different between the two groups.

Asthma Triggers

Certain asthma triggers other than passive smoking such as recurrent respiratory infections, persistent exposure to known irritants, air pollution, cold air, exercise and emotional stress were assessed between the two groups. None of the variables showed a significant difference in prevalence between passive smokers and control group (Table2). Of these variables, recurrent respiratory infections were more prevalent in both groups of asthmatics.

Table 1. Descriptive characteristics of subjects of passive smoker and control group

Variables	**Passive smoker group**	**Control group**	***P*-value**
Age (Years)	5.59± 5.61	7.44± 7.5	0.65
Age of onset(Years)	4.27 ± 1.92	5.12 ± 3.6	0.34
Sex (Male/Female)	27/23	26/24	0.19
Allergic history	12 (24%)	15 (30%)	0.22
Low birth weight	13.3%	15%	0.79
Caesarean section	45.3%	30%	0.27

Table 2. Asthma triggers and family history in patients of the study

Other Triggers			
odors	35%	41%	0.71
Respiratory Infections	58%	49%	0.84
Air pollutions	34%	40%	0.30
Cold air	22%	24%	0.07
Exercise	14%	17%	0.72
Emotional stress	25%	25%	0.42
Pets with fur	18%	21.5%	0.12
pollen	13.5%	15.4%	0.23
Family history of asthma			
Maternal	3 (6%)	2 (4%)	0.44
Paternal	4 (8%)	3 (6%)	0.59

Table 3. Asthma severity according to GINA classifications

Groups	FEV1≥80% Mild persistent/ intermittent	FEV1 60%-80% Moderate Persistent	FEV1≤60% Severe Persistent
Passive smoker	13 (27%)	21 (43%)	14 (30%)
Control	40 (83%)	7 (17%)	0
Passive smoker group:			
<6 years	4 (8.3%)	8 (16.6%)	5 (10.5%)
6-8 years	5 (10.5%)	7 (14.5%)	5 (10.5%)
8< years	4 (8.3%)	6 (12.5%)	4 (8.3%)
Control group:			
<6 years	11 (23.5%)	3 (6.3%)	0
6-8 years	15 (32%)	2 (4.2%)	0
8< years	14 (30%)	2 (4.2%)	0

FEV1: forced expiratory volume

Asthma Severity

Patients were classified based on Spirometry according to GINA classifications. Severe (p=0.001) and moderate (p=0.013) asthma was significantly higher in passive smoker group compare to control (Table 3). Also mild asthma was significantly higher in control group compare to passive smoker group (p=0.002). In passive smoker group, in patients less than 6 years of age, percentage of patients with moderate persistent asthma (16.6%) were significantly higher than mild (8.3%) (*p*=0.02) or severe (10.5%) (*p*=0.034).However, in patients of 6-8 years, percentage of patients with mild (p=0.22), moderate (p=0.43) and severe (p=0.27) asthma were not significantly different in passive smoker group. In patients older than 8 years also, there was no significant differences in mild (p=0.077), moderate (p=0.54) and severe (p=0.61) asthma. However, in patients older than 6 years of age there were no significant differences in severity of asthma (p>0.05).

Cotinine Level and Smoking

Cotinine level was measured in serum, saliva and urine in both groups of patients. Serum cotinine was significantly higher in passive smoker group (6.34±0.9) compared to control group (3.96±1.08) (*p*=001). Saliva cotinine was significantly higher in passive smoker group (4.45±1.33) compared to control group (2.08±1.1) (*p*=0.001).Urine cotinine was significantly higher in passive smoker group (108.67±22.23) compared to control group(41.47±17.16) (*p*=0.0014) (Figure1). The mean and standard deviation of cotinine in all three samples are depicted in Figure 1.

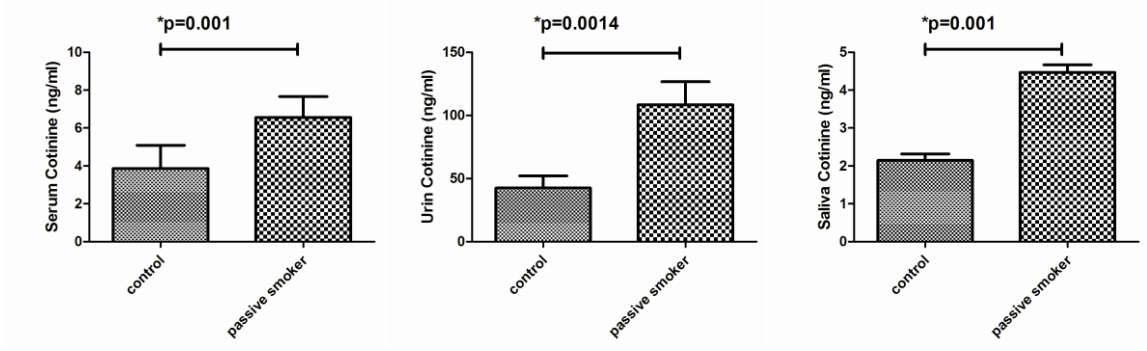

Figure 1.Cotinine levels in serum, urine and saliva are compared between passive smoker and control patients. Bars show mean± standard deviation.

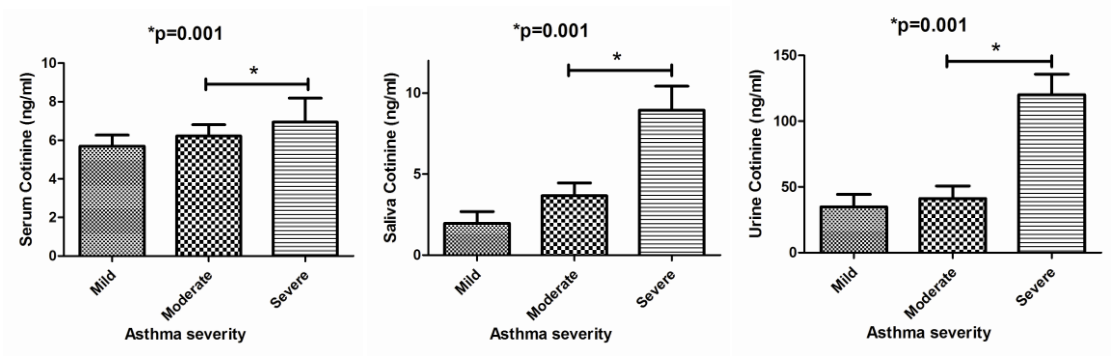

Figure 2. Serum, urine, and saliva cotinine levels in patients with mild, moderate, and severe asthma.

In passive smoker group, serum cotinine levels were significantly higher in severe asthma compared to moderate (p=0.036) and mild asthma (p=0.021). Saliva cotinine levels were significantly higher in severe asthma compared to moderate (p=0.025) and mild asthma (p=0.007). Urine cotinine levels were significantly higher in severe asthma compared to moderate (p=0.005) and mild asthma (p=0.0001) (ANOVA test) (Figure2).

Multivariate regression analysis showed that urine, saliva and serum cotinine levels are independent predictors of severity of asthma. Urine cotinine was associated with significantly increased risk for severe asthma (odds ratio 3.56(95% confidence interval = 1.29-5.53, p=0.01). Serum cotinine (OR: 1.81, 95% CI: 1.35-2.32, p=0.024) and saliva cotinine (OR: 1.66, 95% CI: 1.23-1.98, p=0.031) were also significantly associated with higher risk of severe asthma (Table4).Other possible risk factors were not significantly associated with increased risk of severe asthma (p>0.05) (Table4).

In spearman correlation test, years of exposure to cigarette were not significantly correlated to cotinine levels in serum (r=0.19, p=0.9), urine (r=0.14, p=0.87) and saliva (r=0.12, p=0.99). Age of patients were not also correlated to cotinine levels in serum (r=-0.13, p=0.79), urine (r=0.18, p=0.27) and saliva (r=0.31, p=0.75). Age of onset of symptoms were not also correlated to cotinine levels in serum (r=0.26, p=0.45), urine (r=0.26, p=0.17) and saliva (r=0.22, p=0.37). However, scale of parents cigarette smoking (pack/years) was significantly correlated to serum cotinine (r=0.067, p=0.001), urine (r=0.40, p=0.01), and saliva (p=0.31, p=0.03) (Figure3).

Table 4. Predictors of severe asthma in regression analysis in our patients

Risk factors	OR(95% CI)	*P*value
Serum cotinine	1.81 (1.35-2.32)	0.024
Urine cotinine	3.56 (1.29-5.53)	0.01
Saliva cotinine	1.66 (1.23-1.98)	0.031
Age	1.44(0.75-1.62)	0.47
Age of symptomonset	1.05 (0.48-1.22)	0.33
Allergic history	1.74 (0.54-1.82)	0.068
Low birth weight	0.84 (0.65-1.53)	0.28
Caesarean section	0.64 (0.39-1.37)	0.076

OR: odds ratio; CI: confidence interval

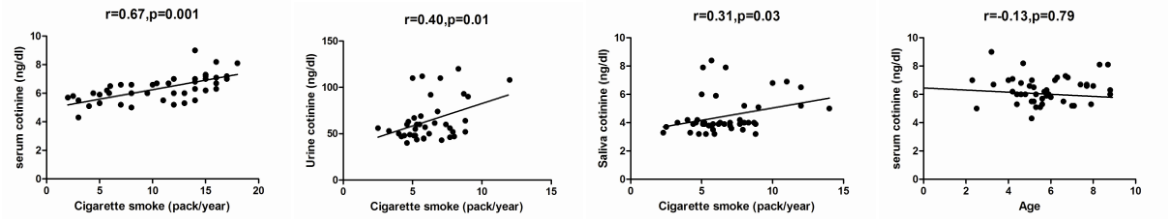

Figure 3. Correlation of serum, urine, saliva cotinine to scale of parents cigarette smoking and age of patients.

DISCUSSION

Cotinine has been a matter of research for a decade. Although passive cigarette smoking is notorious for triggering of asthma symptoms, but its association with asthma severity is not well recognized particularly in children. In this study, we intended to investigate the relationship between asthma severity and cotinine levels in children. Our results showed that there was strong association between serum, urine and saliva cotinine level and asthma severity in children when they were passive smokers.

Our results revealed that the mean serum, urine and saliva cotinine levels were higher in passive smoker group than in control group. Moreover, the odds ratio indicated that higher serum, urine and saliva cotinine levels increased the risk of developing severe asthma in passive smoker children. Association of cotinine with asthma severity has been a matter of uncertainty in many studies. Some reported that increased cotinine levels were significantly associated with decrease in FEV1,[14] peak expiratory flow, and FEF (25-75%)[15]consistent with our study. We have also analyzed Spirometry FEV1 values and cotinine levels which showed a correlation between decrease in FEV1 and increase in cotinine levels. Others have mentioned that passive exposure to smoke in asthmatic individuals is associated with worse lung function, higher number of exacerbations, and greater bronchial hyperreactivity.[16]Our results would help to predict asthma course in children exposed to passive cigarette smoke. If the cotinine levels are high, they may be prone to have severe asthma.

Our results showed that there was no correlation between cotinine levels and age of patients, age of onset of symptoms, or duration of exposure to cigarette. Patients were classified based on age distribution in each class of asthma. In patients less than 6 years of age, moderate persistent asthma (16%) was higher than mild (8%) or severe (10%) asthma. Other studies have shown that among children living in a home exposed to smoking, younger children had significantly higher mean cotinine concentrations than older children.[17] Theoretically, cotinine levels should be associated with duration of exposure. Therefore, if the child is exposed to cigarette for a longer time, higher cotinine levels and more severe asthma is expected. However, severe asthma was not significantly higher in pediatrics older than 6 compare to younger than 6 years. Besides, age was not a significant predictor for asthma severity in regression analysis. Thus the amount of exposure may be more relevant to asthma severity than the age of patient. In our study the age of our patients was not effective in severity of asthma. Cotinine levels should be sought in association with scale of exposure. In other words, if exposure occures around the time of sampling, the cotinine level would be high. Serum cotinine was below 5 ng/ml in control patients (not passive smokers), but heavy passive exposure could result in levels greater than or equal to 10 ng/mlin our study. Urine cotinine levels greater than 100 ng/ml are probably the result of regular active smoking.[18]This wide difference gives a strong association between cotinine level and degree of passive exposure to cigarette smoke. Other results consistent with us showed that cotinine significantly increased with cigarettes smoked per day, and years of exposure,[19]and even cotinine concentration was associated with the number of cigarettes smoked per day.[20]Altogether, with regards to our results, cotinine levels are prominently reflection of degree and dose of exposure instead of duration of exposure to cigarette smoking and could be a valuable marker in following-up of patients exposed to spassive smoke.

One limitation of our study was related to our study design which could not confirm the association of cotinine level with severity of asthma. In future studies, cigarette exposure should be dismantled in passive smoker children and then cotinine level should be measured in order to confirm its association with asthma severity.

In conclusion, cotinine levels were higher in passive smokers compared to non-passive smokers. Besides, cotinine was predictive risk factor for severe asthma. In addition, cotinine levels could be useful in assessing the degree of exposure to cigarette smoke.

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