Original Article

Urinary Cotinine Level in Indonesian Children Exposed to Domestic Cigarette Smoke

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Abstract

Background: Cotinine is a major metabolite of nicotine, and its urinary level is an indicator of exposure to cigarette smoke. The present study was aimed at identifying the urinary cotinine level in Indonesian children who were exposed and not exposed to domestic cigarette smoke. **Methods:** The study was a cross-sectional study in elementary school-aged children who had not smoked. The subjects were categorized into an exposed group and unexposed group based on their exposure status. Data were obtained from a questionnaire and random urinary samples measured using enzyme-linked immunosorbent assay. **Results:** There were a total of 128 subjects, including 64 children in the exposed group and 64 children in the unexposed group. The median level of cotinine in all subjects was 17.95 ng/ml (with a range of 0.1–158.3 ng/ml). The urinary cotinine level in the exposed group was higher than the unexposed group (median: 30.1 ng/ml vs. 8.45 ng/ml; $P < 0.000$). There was a correlation between urinary cotinine levels in children exposed to cigarette smoke and the number of cigarettes smoked by the smokers at home ($P < 0.05$). The optimal cut-off points of urinary cotinine levels in children, which was utilized to evaluate cigarette smoke exposure, was 17.95 ng/ml (81% sensitivity; 81% specificity; $P < 0.000$). **Conclusion:** The urinary cotinine level in children exposed to cigarette smoke is higher than children who are not exposed to domestic cigarette smoke. The urinary cotinine level can be used as a noninvasive marker to evaluate cigarette smoke exposure in children.

Keywords: Children, cigarette smoke exposure, urinary cotinine

Introduction

Exposure to domestic cigarette smoke is an important health problem, as more than 85% of smokers in Indonesia are smoking while their family members at home. Over 97 million Indonesian citizens are exposed to cigarette smoke every day, and 43 million of them are children.[1] Environmental cigarette smoke includes mainstream smoke, which is directly inhaled by a smoker, and sidestream smoke, which is the smoke produced as a result of lighting a cigarette.[2] A major source of children's cigarette exposure occurs at home, potentially leading to health problems.[2]

Cotinine is a major metabolite of nicotine which has more stable characteristics, such as a longer half-life (i.e., approximately 16 h and the urinary excretion is about 10%–15%).[3] Cotinine can be detected by measuring levels in bodily fluids such as blood, urine, and saliva and serves as a marker of environmental cigarette smoke exposure in >80% of the nonsmoker population. The examination of urinary cotinine level is preferable as it is not invasive and is more practical compared to the examination of cotinine serum levels. Until now, there has been no data in Indonesia associated with the urinary cotinine level in passive smokers, including children who are exposed and unexposed to domestic cigarette smoke. This study aimed to identify urinary cotinine levels in children based on exposure to domestic cigarette smoke, as well as its affecting factors, and the cut-off point of urinary cotinine level used to evaluate cigarette smoke exposure in children.

Methods

A cross-sectional study in elementary school-aged children at Cijantung 07 Elementary School, East Jakarta, was conducted between March and July 2014. The study protocol was approved by the Ethical Committee of the Faculty of the Medicine University of Indonesia, Jakarta, Indonesia (Ethical Clearance Number: 689/H2.F1/ETIK/2013). Subjects were

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categorized into exposed and unexposed groups based on their exposure status. A child was categorized as having exposure to domestic cigarette smoke if there was a smoker who lived in the same house as the child. The inclusion criteria were children aged 6–12 years, nonsmokers, and both children and their parents were willing to participate in the study. The exclusion criteria were children who take anti-epileptic and anti-tuberculosis agents, as the treatments may affect cotinine metabolism resulting in urinary cotinine levels of >200 ng/ml. The subjects were recruited using a consecutive sampling method, in which every child who met the inclusion criteria was involved until we achieved the required sample number.

Data collection included height and body weight measurements for calculating body mass index for age (5–19 years) based on the World Health Organization growth reference, filling out a questionnaire to obtain data on identity and sociodemographical characteristics of the subjects, data associated with characteristics of domestic cigarette smoke exposure and history of consuming food that contained nicotine in the last 36 h. Subjects were then given a urinary container and asked to collect their urine for a random urine analysis. The enzyme-linked immunosorbent assay (ELISA) method was used to determine the level of urinary cotinine, using a BQ 096D cotinine kit at the laboratory of the Department of Pulmonology and Respiratory Medicine, Persahabatan Hospital, a National Reference Hospital for Respiratory Diseases in Indonesia.

The obtained data were subsequently analyzed by univariate, bivariate, and multivariate analyses using Statistical Package for Social Science (SPSS) software program version 16.0 (IBM Corp, Armonk, NY, USA). An abnormal data distribution obtained in this study was presented as a median (minimal–maximal). The value of $P < 0.05$ was statistically significant.

Results

The population study was conducted in children aged 6–12 years at Cijantung 07 Elementary School between March and July 2014. The number of obtained subjects was 128 children, which was consistent with the calculated sample size. Subjects were categorized into two groups based on their exposure status to cigarette smoke (i.e., there were 64 children in both the exposed and unexposed groups). The mean age was 9.8 ± 1.6 years, and the median age was 10 years. There were more smoke-exposed males and unexposed females. However, we found no significant association between gender and smoke exposure status ($P = 0.367$), In terms of nutrition status, most of the subjects were normal nutrition status both in the exposed group and in the unexposed group. There was no significant association between nutrition status and cigarette smoke exposure status ($P = 0.279$). Most children have parents with an education level of Junior High or Senior High graduate. There was no significant association between education of father and mother of subject and cigarette smoke exposure status in the study ($P = 0.083$). Subject characteristics based on sociodemographic data can be seen in Table 1.

Subject characteristics, based on exposure to cigarette smoke in children who were exposed to cigarette smoke, showed that there were 17 children (26.6%) who lived with ≥2 smokers. 47 of the children (73.4%) lived in the same house as one smoker. Most children (65.6%) were exposed to the cigarette smoke of smokers who had <10 cigarettes daily. The type of cigarettes smoked by smokers who lived with the children were mostly kretek cigarettes (90.6%, cigarettes made with a blend of tobacco, cloves, and other flavors). The longest duration of children's cigarette exposure was ≥5 years, which was found in 62.5% of the subjects. The mean duration of daily cigarette smoke exposure was >1 h/day, which was found in 26 children (40.6%). For 18 children (28.1%), the last exposure to cigarette smoke in was between 1 and 6 h before. The characteristics of cigarette smoke exposure in the exposed children can be seen in Table 2.

Urinary cotinine levels in children exposed and unexposed to domestic cigarette smoke

The measurement of urinary cotinine level in this study was performed using the ELISA method. The urinary cotinine level

Table 1: Sociodemographical characteristics of the subjects

Variables	Total ($n=128$), n (%)	Exposed group ($n=64$), n (%)	Unexposed group ($n=64$), n (%)	P (χ^2)
Sex				
Male	51 (39.8)	28 (43.8)	23 (35.9)	0.367
Female	77 (60.2)	36 (56.2)	41 (64.1)	
Nutrition status				
Severe thinness - thinness	20 (15.6)	10 (15.6)	10 (15.6)	0.279
Normal	83 (64.8)	38 (59.4)	45 (70.3)	
Overweight - obesity	25 (19.5)	16 (25.0)	9 (14.1)	
Father's education				
Primary school	11 (8.6)	9 (14.1)	2 (3.1)	0.083
Middle-high school	95 (74.2)	44 (68.8)	51 (79.7)	
University or over	22 (17.2)	11 (17.2	11 (17.2)	
Mother's education				
Primary school	16 (12.5)	9 (14.1)	7 (10.9)	0.864
Middle-high school	96 (75)	47 (73.4)	49 (76.6)	
University or over	16 (12.5)	8 (12.5)	8 (12.5)	

had abnormal data distribution [Supplementary Figure S1]; therefore, nonparametric tests were utilized using a median value. The median of the cotinine level was 17.95 ng/ml. The lowest level was 0.1 ng/ml and the highest level was 158.3 ng/ml. There was a significant cotinine level difference between children exposed and unexposed to cigarette smoke ($P = 0.000$). The cotinine level in both groups can be seen in Figure 1.

In this study, we found two subjects who had extreme cotinine levels, 154 ng/ml and 158.3 ng/ml. Subjects who had extreme levels were then confirmed through a direct interview with the subjects, their parents and family. It was also confirmed that both subjects were nonsmokers. There were no cotinine levels that exceeded >200 ng/ml; therefore, no subject was excluded from the study.

Factors affecting urinary cotinine levels in children exposed to domestic cigarette smoke

In the group of children who were exposed to domestic cigarette smoke, we found that the median of urinary cotinine in boys was 29.5 ng/ml, whereas it was 30.2 ng/ml in girls. There was no significant urinary cotinine level difference between boys and girls ($P = 0.882$). In this study, we also did not find a significant correlation between urinary cotinine levels in children exposed to cigarette smoke and the level of education of their fathers ($P = 0.632$) and mothers ($P = 0.792$).

The present study did not show a significant correlation between age and urinary cotinine level in children exposed to domestic cigarette smoke ($P = 0.845$). A factor associated with urinary cotinine levels was the number of cigarettes smoked by smokers who lived in the same house as the child ($P < 0.05$) can be seen in Table 2.

Table 2: Urinary cotinine level in the exposed children based on exposure characteristics and nicotine diet

Variable	Total (%)	Median (minimum-maximum)	*P*
Number of household smokers			
≥2 smokers	17 (26.6)	36.8 (7.9-154.0)	0.230*
1 smoker	47 (73.4)	29 (4.1-158.3)	
Number of cigarettes per day			
>20 cigarettes/day	2 (3.1)	76.15 (49.6-102.7)	<0.05#,†
10-20 cigarettes/day	20 (31.2)	63.15 (7.7-158.3)	
<10 cigarettes/day	42 (65.6)	25.4 (4.1-106.3)	
Cigarette's type			
Kretek	58 (90.6)	27.0 (4.1-158.3)	0.314#
White cigarettes	3 (4.7)	29.8 (29-86)	
Mixed	3 (4.7)	62.3 (32.6-103.3)	
Duration of exposure (years)			
≥5	40 (62.5)	29.5 (4.1-126.0)	0.475*
<5	24 (37.5)	30.4 (12.6-158.3)	
Tobacco smoke exposure per day (h)			
>1	26 (40.6)	40.85 (7.7-158.3)	0.189*
≤1	38 (59.4)	27.4 (4.1-154)	
Last cigarettes smoke exposure (h)			
<1	13 (20.3)	29.8 (12.6-120)	0.575#
1-6	18 (28.1)	28.0 (10.6-102.7)	
7-12	16 (25.0)	63.15 (7.7-154)	
>12	17 (26.6)	29.0 (4.1-158.3)	
Nicotine diet			
Yes	35 (54.7)	25.8 (7.7-158.3)	0.632*
No	29 (45.3)	30.7 (4.1-154)	

†*Post hoc* Mann-Whitney test: ≥20 cigarettes/day versus 10-20 cigarettes/day P=0.732; 10-20 cigarettes/day versus <10 cigarettes/day P<0.05; ≥20 cigarettes/day versus <10 cigarettes/day; P<0.05, *Mann-Whitney test, #Kruskal-Wallis test

Cut-off point analysis on urinary cotinine level

A receiver operating characteristic (ROC) curve analysis was performed to obtain a cut-off point for urinary cotinine levels in children. The cut-off point was determined to evaluate the sensitivity and specificity of urinary cotinine level in differentiating children who were exposed and unexposed to domestic cigarette smoke. In Figure 2, the optimal cut-off point was found at a urinary cotinine level of 17.95 ng/ml, with 81% sensitivity and 81% specificity. The value of area under curve (AUC) was 91.2% (95% confidence interval (CI): 86.4%–96.1%) and it was statistically significant ($P = 0.000$). A urinary cotinine level of ≥17.95 ng/ml indicated that the child was exposed to domestic cigarette smoke.

Discussion

The present study is a cross-sectional study conducted to identify urinary cotinine levels in children who are exposed and unexposed to domestic cigarette smoke, as well as the affecting factors, and to identify the cut-off point of urinary cotinine levels in children to evaluate cigarette smoke exposure. A urinary cotinine examination is preferred as it is less invasive and more practical for children. The level of cotinine in the group exposed to cigarette smoke was higher than in the unexposed group (median: 30.1 vs. 8.45 ng/ml; $P < 0.000$). The results of the present study are consistent with the results of a study by Bono *et al*. in their study, conducted with 14-year-old teenagers, significant urinary cotinine levels in children exposed to cigarette smoke were obtained (28.4 ng/ml); while in children who were unexposed to cigarette smoke, the cotinine level was 13.4 ng/

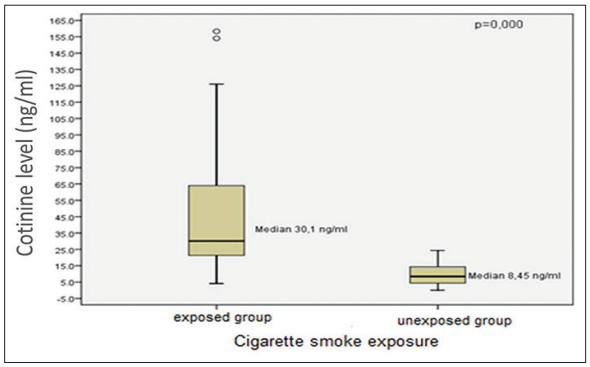

Figure 1: Box-plot distribution of urinary cotinine levels in children based on exposure to domestic cigarette smoke. The urinary cotinine level in children exposed to cigarette smoke was significantly higher than in children unexposed to cigarette smoke ($P < 0.05$)

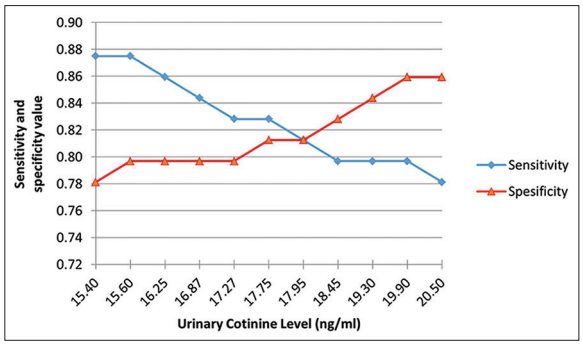

Figure 2: Cut-off curve of urinary cotinine. The cut-off point for urinary cotinine levels, based on a receiver operating characteristic curve analysis, was determined to differentiate children exposed and unexposed to domestic cigarette smoke (i.e.,17.95 ng/ml with 81% sensitivity and 81% specificity)

ml ($P < 0.05$).[4] Results of another study conducted by Yildirim *et al*.(2011) demonstrated, for children aged 4–6 years, that the urinary cotinine level of children exposed to domestic cigarette smoke was 77.6± standard deviation (SD) 41.4 ng/ml and the level in unexposed children was 11.9± SD 2.3 ng/ml ($P < 0.001$).[5] The detected urinary cotinine level in unexposed children may have been caused by the effect of environmental cigarette smoke as a component of air pollution. In this study, the urinary cotinine levels were highly varied, with a large range (0.1–158.3 ng/ml). This may have occurred since everyone has varied absorption, metabolism and elimination of cotinine in the urine. The level also is varied based on amount of cigarette smoke exposure.[6]

The present study is consistent with other study in adult subjects. Study involving adults subjects showed nonsmokers and cigarette smoke-exposed group have higher median urinary cotinine level compared to nonexposed group.[7] In other study, there were strong associations between number of cigarette smoked daily with urinary cotinine level among adult current smokers.[8]

In the present study, we found that a factor affecting the urinary cotinine levels in exposed children was the number of cigarettes smoked by the smoker at home. Similar results were also found by Bono *et al*. who demonstrated a correlation between cotinine levels and the number of cigarettes smoked at home by the family member(s).[4] A study conducted in Germany by Scherer *et al*. in children aged 6–7 years also found similar results, showing that there was a significant difference between mean urinary cotinine/creatinine ratios in children exposed to the cigarette smoke of 5, 6–10, 11–20 and >20 cigarettes per day (29.4 ng/ml, 38 ng/ml, 48.9 ng/ml, 67.8 ng/ml, respectively).[9] A study conducted by Vardavas *et al*., in teenagers aged 13–17 years exposed to cigarette smoke, showed that there is a strong correlation between serum cotinine levels and the number of cigarettes smoked per day ($R^2 = 0.443$; $P = 0.001$).[10] Urinary cotinine levels of children exposed to domestic cigarette smoke indicate that there is no significant difference in the number of smokers in the home, type of cigarettes, duration of exposure, mean exposure, and last exposure. In our multivariate analysis, number of smokers contributed 17.8% toward urinary cotinine level among smoke-exposed children. There may be other contributing factors that are beyond the investigated variables in the current study. A study conducted by Ekebicer suggested that there is no correlation between urinary cotinine levels and mean daily exposure.[11] Different results were obtained by Scherer *et al*. Their study showed that there are significant mean urinary cotinine level differences depending on the number of smokers at home, the number of cigarettes smoked daily and the duration of daily cigarette smoke exposure. The levels of urinary cotinine/creatinine ratios in children exposed to cigarette smoke for 1–2 h, 3–5 h and <6 h daily were 36.9 ng/ml, 41 ng/ml, and 54.7 ng/ml, respectively. In their study, Scherer *et al*. also found urinary cotinine/creatinine ratios in children who lived with 0, 1, 2, and 3 smokers, which were 10.2 ng/ml, 34.4 ng/ml, 45.4 ng/ml, and 56.6 ng/ml, respectively.[9]

In the present study, there was no significant correlation between cotinine levels and age ($P = 0.845$), which can occur at the age of all subjects is within a group of 6–12-year-old elementary school children. Based on the multivariate analysis of linear regression, we found that the number of cigarettes was significantly correlated with urinary cotinine levels in children exposed to domestic cigarette smoke ($P < 0.05$). A study conducted by Mannino *et al*. suggested that a strong predictor of cotinine levels in children is the number of cigarettes smoked at home. Other determining factors are race, ethnic group, children's age, and the size of their house.[12]

Increased urinary cotinine levels in children exposed to domestic cigarette smoke compared to those who are not exposed to domestic cigarette smoke indicates that the nicotine of cigarette smoke is inhaled by the children and metabolized into cotinine. Individuals exposed to cigarette smoke, inhaling nicotine into their body, are faced with health problems like those of active smokers. This may occur since there is no safe threshold for cigarette smoking. Cigarette smoke exposure in children impacts their health in ways that might continue until the children become adults.[2] Parents must understand that cigarette smoke exposure in children may cause various diseases and they are responsible for not endangering their children's' health. Government needs to develop policies that provide information to parents and families about the danger of cigarette smoke exposure in children. Law supports about not smoking in homes or when children are present, in the form of governmental and local policies, are necessary.

Cut-off point of urinary cotinine levels

The use of cotinine levels in plasma, saliva, and urine to differentiate between active smokers, passive smokers and those who are not exposed to cigarette smoke has been largely applied; however, there is no cut-off point that has been generally accepted. In the present study, we found a cut-off point for urinary cotinine levels, based on a ROC curve analysis, to differentiate children exposed and unexposed to domestic cigarette smoke (i.e., 17.95 ng/ml with 81% sensitivity and 81% specificity). The value of AUC was 91.2% (95% CI: 86.4%–96.1%) and it was statistically significant ($P < 0.000$). A urinary cotinine level of ≥17.95 ng/ml indicates that the child is exposed to domestic cigarette smoke. Another cut-off point was obtained in a study conducted by Kim *et al*., in subjects aged > 18 years. The study obtained a cut-off point to differentiate those unexposed and exposed to cigarette smoke (i.e., 9.42 ng/ml with 53% sensitivity

and 57.1% specificity).[13] Different use of the obtained cut-off point of the present study can be caused by different age groups of subjects and a different population. There are varied cotinine levels results due to genetic variations on the CYP2A6 enzyme which is involved in nicotine metabolism. The metabolism process turns nicotine into cotinine. This can affect the level of cotinine in each race. Other factors that can cause different cotinine cut-off points are varied cigarette smoke exposure, varied metabolism and elimination in each individual, as well as physiological factors.[6] The limitation of the present study is that the threshold level of cotinine should be determined by its harmful biological effect rather than by its absorption, which would be more relevant to its health issue in the general population. Other limitation was limited sample size which involved 64 smoke-exposed children. To the best of our knowledge, however, our study was the first to report urinary cotinine level in Indonesian children.

Conclusion

The present study found a significant urinary cotinine level difference between the exposed and unexposed children. A factor that affects the urinary cotinine levels in exposed children are the number of cigarettes smoked by the smoker at home. The evaluation of urinary cotinine levels can be used as a noninvasive marker for exposure to cigarette smoke and is a practical examination method for children. The main source of cigarette smoke exposure in children is their environment at home. Stipulations should be put into effect to create smoke-free zones in homes so that the deleterious impacts of cigarette smoke on children's health can be prevented. Hospital-based studies are required to evaluate cigarette smoke exposure, using cotinine levels, for children with respiratory complaints. Studies with cohort design involving more subjects are also necessary to evaluate the urinary cotinine levels in children who are at risk for developing respiratory complaints.

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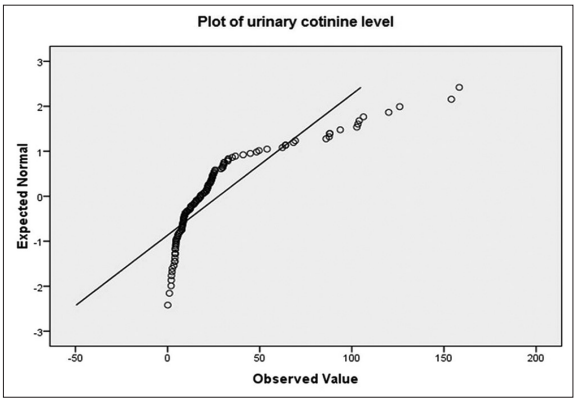

Supplementary Figure S1: Scatter plot data that described the abnormal distribution of urinary cotinine level in smoke-exposed Indonesian children

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Conflicts of interest

There are no conflicts of interest.

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