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Original Article

Cotinine and interferon-gamma levels in pre-school children exposed to household tobacco smoke

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Abstract

Background Environmental tobacco smoke has been consistently linked to negative health outcomes, especially in children, including an increased susceptibility to infections. Cigarette smoking has a depressive effect on interferon-γ (IFN-γ). Serum cotinine is a marker of exposure to smoke.

Objective To determine the association between serum cotinine and interferon-γ (IFN-γ) levels in children with household tobacco smoke exposure.

Methods We conducted a cross-sectional study at the Tumumpa and Singkil Districts of Manado, Indonesia, from February to May 2012. Subjects were collected by consecutively sampling of healthy children aged 1-3 years who came to the integrated health posts. Seventy-four children were recruited and consisted of two groups of 37 subjects each, the tobacco smoke exposure group and the non-tobacco smoke exposure group. Blood specimens were collected from all subjects for laboratory blood tests of cotinine and IFN-γ levels. Results were analyzed by T-test and Pearson's correlation analysis with a $P<0.05$ is considered as statistically significant.

Results There was no significant correlation between serum cotinine and interferon-γ levels in the tobacco smoke exposure group. However, the interferon-γ level in the tobacco smoke exposure group was significantly lower than that of the non-tobacco smoke exposure group ($P<0.0001$).

Conclusion Cotinine is not related to the interferon-γ level in children exposed to tobacco smoke, however, the interferon-γ level in children with tobacco smoke exposure is lower than in the non-tobacco smoke exposure group.

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Keywords*: environmental tobacco smoke, nicotine, IFN-γ, pre-school children*

Since the mid-1980s, there has been increased interest in the effects of passive smoking on children's health. As children spend much of their early lives in the presence of their parents, children whose parents smoke will have a prolonged and close exposure to environmental tobacco smoke. Environmental tobacco smoke has been consistently linked to negative health outcomes, especially in children, including an increased susceptibility to infection.[1-3] Cigarette smoke is a complex and dynamic mixture of microscopic particles and gases, comprised of over 4,000 chemicals, very few of which have actually been assayed for immunosuppressive activity.[4]

Cotinine has become increasingly accepted as a short-term marker of exposure to smoke because of its relatively long half-life, approximately 20 hours compared that of approximately 2 hours for nicotine. It also less susceptible to fluctuatuions during exposure to tobacco smoke and can be conveniently measured in blood, urine, and saliva.[16] Plasma cotinine concentration is the most widely used and validated

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biomarker for environmental tobacco smoke or secondhand smoke and reflects exposure to nicotine largely from the previous 1 to 2 days. Plasma cotinine levels lower than 15 mg/mL (85 nmol/L) is considered to be exposure to environmental tobacco some in the agsence of smoking.[5,6]

The depressive effects of cigarette smoking on interferon-gamma (IFN-γ), was confirmed by an in vivo study that found decreased IFN-γ levels in children whose parents smoked compared to that of children of non-smokers.[3] IFN-γ is a cytokine produced primarily by activated CD4+ or CD8+ T cells and natural killer cells, and is recognized as a chief mediator of innate as well as adaptive immunity. IFN-γ is an important cytokine in the host defense against viral and microbial pathogens. It induces a variety of physiologically significant responses that contribute to immunity. Among the biological activities of IFN-γ is macrophage activation.[7-9]

There have been conflicting reports on the effects of nicotine on cytokines. Previous studies revealed that nicotine, one of the main constituents of cigarette smoke, disrupts the differentiation and functional properties of monocyte-derived dendritic cells, leading to decreased Th1 cytokine production, such as IFN-γ. There have been few studies on the effect of tobacco smoke exposure in Indonesian pre-school children, especially in Manado.

The aim of this study was to assess the association between serum cotinine and interferon-γ (IFN-γ) levels in children with household tobacco smoke exposure.

Methods

We conducted a cross-sectional study at the Tumumpa and Singkil Districts of Manado from February to May 2012. A simple random sampling method was used to select 4 out of the 48 integrated health posts in the districts. Information on parental smoking was collected at the time of enrollment. Smokers were either mothers or fathers of the children, both parents, or any primary caregivers, usually grandmothers, grandfathers or other relatives. Parents were asked to clearly specify when the caregivers started smoking, how many cigarettes were smoked per day, and how many of these were smoked in the presence of the children. Serum cotinine was used to confirm the tobacco smoke exposure in children.

Children were classified as exposed to tobacco smoke if they had been exposed to the smoke of one or more cigarettes per day and had a plasma cotinine level of 1-10 ng/mL. Children with cotinine levels of <1 ng/mL were considered as not exposed to tobacco smoke.

Subjects' parents provided informed consent. Sampling was conducted consecutively in children aged 1-3 years at the chosen integrated health posts, resulting in 37 children who were exposed to cigarette smoke and 37 children who were not exposed to cigarette smoke.

The inclusion criteria were healthy children without malnutrition. We excluded children with allergies, tuberculosis and acute diseases.

We collected 10 mL-blood specimens from all subjects for laboratory blood tests. Serum cotinine levels were measured with the *Cotinine Elisa* kit, produced by *Bio Quant D&R Kits, San Diego, California*. Interferon-γ levels were measured with the *Human Interferon γ – High Sensitive Elisa Kit®* produced by *eBioscience*, Vienna, Austria. Data were analyzed with T-test and Pearson's correlation analysis with a significance level of $P<0.05$.

Results

A total of 74 children were enrolled in this study, with 48 females and 26 males. The mean age of children in the tobacco smoke exposure group was 2.62 (SD 0.80)

Table 1. Mean IFN-γ levels in subjects exposed or not exposed to tobacco smoke

Study groups	Mean IFN-γ level (SD), pg/mL	95%CI	P value
Tobacco smoke exposure	0.32 (0.20)	0.25 to 0.39	< 0.0001
Non-tobacco smoke exposure	2.56 (1.28)	2.13 to 2.99	

years, and that of the non-tobacco smoke exposure group was 2.70 (SD 0.85) years.

The mean cotinine level in subjects exposed to tobacco smoke was 1.77 (SD 0.47) ng/mL. There was no significant correlation between cotinine level and IFN-γ level in subjects exposed to tobacco smoke (r= -0.05; P=0.37). (Data not shown.) However, there was a significantly lower mean IFN-γ level in the group exposed to tobacco smoke than in the group not exposed to tobacco smoke, (P<0.0001) (**Table 1**).

Discussion

Passive smoke affects up to 80% of the general population and like active smoking, is harmful. Exposure to tobacco smoke has an enormous adverse impact on infants' and children's health. Without question, they are the most vulnerable at-risk population.[10,11] Exposure of children to environmental tobacco smoke in the home increases the risk of middle ear disease, asthma, wheezing, cough, phlegm production, bronchitis, bronchiolitis, pneumonia, and impaired pulmonary function. It has also been associated with snoring, adenoid hypertrophy, tonsillitis, and sore throats. The incidence of tonsillectomies doubled for children who live in households with smokers.[2,12]

Smoking during pregnancy seems to add an additional risk to that associated with postnatal exposure to environmental tobacco smoke. Maternal smoking during pregnancy has been associated with an increased risk for infant death as a result of respiratory disease.[13,14]

The mean cotinine level in our subjects exposed to tobacco smoke was 1.77 (SD 0.47) ng/mL. A lower range of cotinine levels were found in a previous US study. Marano *et al.*[15] and Yolton *et al.*[16] measured plasma cotinine levels in children and adolescents exposed to tobacco smoke and found them to be 0.12 ng/mL[15] and 0.23 ng/mL,[16] respectively.

The higher cotinine level in our study may reflect greater exposure to tobacco smoke. Also, our study was undertaken in a population who resided in multi-unit housing with poor ventilation. Ventilation rates may influence cotinine levels.[5]

In our study, we found no significant correlation between plasma cotinine and IFN-γ levels in the group of children exposed to tobacco smoke, similar to results from a previous study.[17]

Two previous in vitro studies showed that hydroquinone had a much greater immunosuppressive effect, compared to that of nicotine, as well as other components in cigarette smoke. One of the immunosuppressive effects was low production of IFN-γ. Hydroquinone is a major phenolic compound in cigarette tar that, along with catechol and phenol, is released from the tobacco by pyrolysis of plant flavinoids.[18,19]

Another in vitro study by Vassalo *et al.*[18] found that cigarette smoke extract suppressed human dendritic cell function, leading to preferential induction of Th2. In contrast to cigarette smoke extract, nicotine did not inhibit dendritic cell-induced T-cell priming. However, Guinet *et al.*[19] noted that nicotine significantly changed dendritic cell functional characteristics at 200 μg/mL.

We found a significant difference in IFN-γ levels between the two groups, with lower IFN-γ level in the tobacco smoke exposure group than in the non-tobacco smoke exposure group (P<0.0001). Similarly, Tebow *et al.*[3] found lower IFN-γ levels in children exposed to tobacco smoke, as well as increased frequency of respiratory tract infections.[3] Lower IFN-γ levels may have an immunosuppressive effect, hence, increasing the frequency of infection.[3,7]

It has been widely hypothesized that cigarette smoke-induced aberrance in dendritic cell function is an important mechanism by which smokers develop cancer, infection, and allergies. Cigarette smoke extract was shown to inhibit dendritic cell-mediated priming of T cells, specifically inhibiting the secretion of IFN-γ while enhancing the production of IL-4.[19,20]

A limitation of this study was that we did not take into account deficiencies in IFN-γ receptors that may influence IFN-γ levels. Another limitation was that we did not determine other cigarette smoke component levels that may also influence IFN-γ production.

In conclusion, we find that nicotine level in children exposed to household tobacco smoke is not associated with IFN-γ level. However, IFN-γ level in children with tobacco smoke exposure is lower than in children without tobacco smoke exposure.

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