Investigation of serum surfactant protein a and d levels in children exposed to cigarette smoke

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Received - 03 September 2018 Initial Review - 27 September 2018 Aceepted - 12 October 2018

ABSTRACT

Background: Depending on the degree of exposure to cigarette smoke, various health problems can emerge in children. It is needed to have biochemical data of passive smoking to define the risks and to count the benefits of anti-smoking responses. **Objective:** The objective of the study was to evaluate the effect of smoke exposure on the surfactant protein (SP) A and D by measuring the cotinine level in the lungs of the children who are exposed to passive cigarette smoke. **Methods:** This case–control study was conducted between December 2012 and September 2013. İn this study, total 79 children were included who were admitted to the general pediatric outpatient clinic of a medical university. Out of them, 51 children were exposed to cigarette smoke and 28 children were not exposed to cigarette smoke. In a survey was applied to evaluate the smoke exposure, and urinary cotinine levels were measured. Cotinine level was measured by chemiluminescence method (children's urines are used), and serum SP-D and SP-A levels were measured by ELISA method (peripheral venous blood is used). **Results:** The average urinary cotinine level of the children who were exposed to smoking was 622.27±600.66 ng/ml and 4.25±7.50 ng/ml of the children who were not exposed. The mean serum SP-A level was high (2.64±0.78 U/L) in children exposed to smoking than that in non-exposed children (2.2±0.76 U/L) and this difference was statistically significant ($p<0.001$). The serum SP-D level was high in children who were exposed to smoking, but it was not statistically significant. It was verified that there was a correlation between the average urinary cotinine level and serum SP-A level ($r=0.257$, $p=0.02$) but it was not true for SP-D level. **Conclusion:** We found that the serum SP-A level, which has a big role on lungs' natural immune system, is higher in the children who are exposed to smoking when compared to the non-exposed children. This indicates that cigarette's inflammatory effect increases as a response to its anti-inflammatory effect in the serum level.

Key words: *Child, Cotinine, Exposure to smoke, Surfactant proteins D, Surfactant proteins A*

Exposure to cigarette smoke is one of the most discussed topics today, with its many aspects ranging from medical outcomes to social and legal aspects. Problems of passive smoking, such as an increased risk for the incidence of sudden infant death and respiratory tract diseases such as decreased pulmonary function, pneumonia, bronchitis, and otitis media, are among current topics of discussion [1,2].

Four major proteins are reported that are functionally important and form a surfactant compound. These are called surfactant proteins (SP) A, B, C, and D. SP-A and SP-D are influential in the defense system of the lungs, and they are believed to be the components of the immune system that first interact with and identifies a pathogen. They are very effective in binding of the encapsulated bacteria, enhancing of the antibacterial effects of alveolar macrophages, opsonization of viruses, and in an increase of the phagocytosis of pneumocystis carinii. In addition, they regulate the inflammatory response of the lungs and prevent an excessive inflammatory response by suppressing some of the neutrophil functions. They are also important for the use of surfactants which become structurally and functionally impaired over time in the making of new surfactants (recycling) by being taken into Type II cells and for the protection of surfactants from inactivation by inactivating plasma proteins [3,4].

Smoking affects local SP A and D levels negatively [5]. Reduced surfactant material and progressive damage to Type II cells were detected in the lung lavages of rats exposed to cigarette smoke [5]. It has been determined that the frequency of attacks increases in chronic obstructive pulmonary disease (COPD) patients with a high serum SP-D level [5]. There are no studies on the effect of cigarette smoking or exposure to cigarette smoke on SP-A and D levels in children as far as we know. The aim of this study was to detect SP A and D levels in children exposed to cigarette smoke.

MATERIALS AND METHODS

This cross-sectional study was conducted between December 2012 and September 2013. This study confirmed to the principles

of the 2008 Declaration of Helsinki and was approved by the local ethics committee (approval number: 20.06.2013/12067). Informed consent was obtained from the parents of the children who were included in the study. A total of 79 children were included in the study who was admitted to the general pediatric outpatient clinic of a medical university. Among them, 51 children were exposed to cigarette smoke and 28 children were not exposed to cigarette smoke. Those who were exposed to environmental cigarette smoke although they did not smoke themselves (belonging to the family where at least 1 cigarette is smoked per day or those with at least 2 h of exposure to environmental cigarette smoke) were identified as passive smokers, and those with no smokers in the family and who were not exposed to cigarette smoke were identified as the control group. The children whose complete blood count, biochemistry, urine analysis, and chest X-ray results were not in normal ranges or children with additional diseases, were excluded from the study.

The data were collected by the researcher, using a face-to-face interview technique. The study was explained to the parents of the children, and they were provided with a questionnaire and informed that blood and urine samples would be collected from their children. The questionnaire contained information such as - the interviewee's degree of kinship to the child, their socioeconomic status, and their demographic information. It was asked whether the mother smoked during pregnancy, whether the father was a smoker and how much he smoked, whether there was any other smoker than the father and the mother in the house and how much they smoked, number of cigarettes smoked per day in the household, how the house was heated, and whether the child or other members of the family had a condition requiring regular check-ups and regular drug use. Urine samples were taken for urine cotinine level with an objective criterion of exposure to cigarette smoke. The body weight and height of every child were measured.

Test principle included the collection of urine samples from all children in sterile and capped urine containers (for urine cotinine levels). At the same time, 5 cc blood samples were collected in heparin-washed tubes to be studied for SP A and D. The collected blood samples were centrifuged for 10 min at 3000 rpm, and the separated serums were stored at −80°C until the date of the study. Assignment of urine cotinine levels was carried out with the enzyme immunoassay method, using the ROCHE HITACHI 912 device. Cotinine levels were calculated in ng/ml. Serum SP A and serum SP D levels were measured with the ELISA method, using the "BioVendor Research and Diagnostic Products" brand kit.

RESULTS

A total of 79 children were included in the study, and they were divided into two groups based on cigarette smoke exposure. The demographic and anthropometric data for both the groups are given in Table 1. The difference between the groups in terms of sex ratios (p=0.56) as well as in terms of age, weight, height, and body mass index values p>0.05 was not statistically significant.

The mean urine cotinine level was statistically significantly higher in the passive smoker group compared to the control group (p<0.001). The mean serum SP-A level and the serum SP-D level were higher in passive smoker group than the control group was higher in the group exposed to cigarette smoke compared to the non-exposed group, this was not statistically significant (p=0.067) (Table 2). There was a correlation between the mean cotinine level and SP-A level of the group exposed to cigarette smoke (r=0.257, p=0.02). No correlation was found for SP-D (r=−0.06, p=0.67). Children's mean urine cotinine level and SP-A and SP-D values according to exposure to cigarette smoke are given in Table 2.

SP-A and D levels were higher among those who were in an environment where >10 pcs/day of cigarettes were smoked compared to those who were in an environment where <10 pcs/day of cigarettes were smoked. The SP-A level was found to be statistically significant, but the SP-D levels were not statistically significant (Table 3).

DISCUSSION

Exposure to cigarette smoke is one of the most discussed topics today, with its many aspects ranging from medical outcomes to sociological and legal aspects. When this topic is handled in terms of children's exposure, it is observed that children are usually exposed to cigarette smoke due to close family members who smoke. Depending on the degree of exposure to cigarette

Table 1: Comparison of the mean age, weight, height, and BMI values of the groups

Parameters Tested	Exposed to cigarette smoke, n: 51	Not exposed to cigarette smoke n: 28	p[b]
Sex (M/F)	25/26	14/14	0.56
Age (years)	2.6±1.79[a]	2.3±1.77[a]	0.80
Weight (kg)	10.7±3.7[a]	10.5±4.5[a]	0.93
Height (cm)	84.13±15.12[a]	82.13±18.23[a]	0.82
BMI (kg/m^2)	14.8±0.85[a]	15.02±0.52[a]	0.34

[a]Given as mean±SD. [b]The Mann–Whitney U test was used. BMI: Body mass index, SD: Standard deviation

Table 2: Urine cotinine, SP-A, and SP-D values of the groups

Parameters Tested	Exposed to CS, n: 51	Not exposed to CS, n: 28	p[b]
SP-A (U/L)	2.64±0.78[a]	2.2±0.76[a]	0.033
SP-D (U/L)	13.5±3.6[a]	12.1±2.8[a]	0.067
Cotinine (ng/ml)	622.27±600.66[a,b]	4.25±7.5[a,b]	<0.001

[a]Given as mean±SD. [b]The Mann–Whitney U test was used. SD: Standard deviation, SP: Surfactant protein

Table 3: SP-A and SP-D levels according to the number of cigarettes smoked

Cigarettes	>10 pcs, n: 15	<10 pcs, n: 32	p[b]
SP-A (U/L)	3.49±0.8[a]	2.3±0.45[a]	<0.001
SP-D (U/L)	14.5±3.9[a]	13.2±3.4[a]	0.248

[a]Given as mean±SD. [b]The Mann–Whitney U test was used. SD: Standard deviation, SP: Surfactant protein

smoke, various health problems can emerge in children. The most important of these problems are intrauterine growth restriction in babies, low birth weight, perinatal and neonatal mortality, decreased pulmonary function, bronchitis, pneumonia, asthma, otitis media, neurodevelopmental delay, behavioral problems, decline in school achievement, and sudden infant death [6,7].

SP-A and SP-D, which are among collections, are proteins that contribute to the regulation of the immune and inflammatory responses of the lungs and play an important role in the innate immune system against inhaled microorganisms and allergens. They play a role in protection against both viral, bacterial, and fungal infections, and apoptotic cells [3,4,8]. Intra-alveolar surfactant clearance was significantly reduced in mice with congenital SP-D deficiency (knockout). In this case, surfactant lipids and SP-A and SP-B gradually accumulate in the alveoli, resulting in the activation of alveolar macrophages and the development of peribronchial-perivascular inflammation and emphysema [9,10].

All oxidants, directly and indirectly, cause the formation of free radicals. The presence of a strong oxidant level causes low levels of antioxidants. The formation of low levels of the antioxidants plays a role in the pathogenesis of many diseases. There are many antioxidants in plasma. The TAS has been reported to represent all antioxidants [11,12]. In a study by Shermatov *et al*. [13], passive smoking was observed as a leading cause of DNA damage in children.

It has been found that the particles taken in by smoking interact with the surface film layer of the surfactant and change the active structure by decreasing the maximum surface area. Progressive damage in the Type II cells, from which SP-A and SP-D are secreted, of rats exposed to cigarette smoke and decreased surfactant material in their lung lavages were detected [5]. In our study, the SP-A level was significantly higher in the passive smoking group compared to the control group. While the serum SP-D level was higher in the passive smoking group, this was not statistically significant. To the best of our knowledge, there are no studies evaluating serum SP-A and SP-D levels in children exposed to cigarette smoke. For this reason, we could not compare our results with a similar study. However, a number of previous studies have evaluated bronchoalveolar lavage (BAL) fluid and SP-A and SP-D levels in adult smokers.

In a study by Lomas *et al*. [14], serum SP-D concentrations were higher in smokers compared to non-smokers. El-Deek *et al*. [15] found the serum SP-D levels of COPD patients to be significantly higher compared to the control group. In addition, serum levels of SP-D were significantly higher in smokers with COPD compared to non-smokers with COPD. They also found a significant negative correlation between pulmonary function test results and serum SP-D levels. These data suggest that the serum SP-D level is a promising biomarker for monitoring the progression of COPD [15].

In the study conducted by Ozyurek *et al*. [16], it was determined that exposure to smoking increases the serum SP-D level while decreasing the SP-D level in BAL. SP-D levels were found to be low in the inductive sputum of active smokers who were not using inhaled corticosteroids. This result indicates the importance of airway inflammation in the pathogenesis of COPD. In patients with COPD with a high serum SP-D level, the frequency of COPD attacks was found to have increased within a 6-month period.

Haeberle *et al*. [17] found that SP-A and SP-D levels in the BAL fluid were lower in patients with respiratory syncytial virus (RSV) compared to subjects in the control group. The SP-A, B, and D levels in the BAL fluid collected from more severe RSV patients connected to ventilators were shown to be lower compared to those who were not connected to a ventilator. This severe infection is due to the prolongation of viral persistence in the lungs and shows that SPs are associated with viral clearance [18,19].

The finding that serum SP-A and SP-D levels are highly detected in patients with pulmonary inflammation such as idiopathic pulmonary fibrosis, pneumonia, tuberculosis, submassive pulmonary embolism, and pulmonary damage suggests that pulmonary inflammation spreads systemically circulating SP-A and SP-D released from Type II pneumocytes in the lung [20,21].

Only serum levels were assessed in our study. SP-A and SP-D levels in BAL were not checked because our study involved healthy children and it was ethically not possible to perform a bronchoscopy. In addition to studies that only evaluate serum levels like our study, there are also studies that evaluate both BAL and serum levels in the literature [22].

Removal of excess or inactive surfactants from the alveolar space is possible through pinocytosis by pneumocyte II, other epithelial cells, and macrophages, through being pushed toward the upper respiratory tract with the help of cilium and intra-alveolar pressure, or through passing the alveolar epithelium and capillary endothelium and mixing with blood [23,24]. The fact that SP-A and SP-D are released only from peripheral lung tissue supports the idea that pulmonary inflammation spreads to systemic circulation [23]. In light of this information, the fact that the serum level is associated with the BAL level shows that checking serum levels are adequate for showing SP-A and SP-D levels. For this reason, we believe that our SP-A and SP-D values provide reliable information.

We aimed to look at the correlation between serum SP-A and SP-D levels and the number of cigarettes smoked to evaluate the relationship between the degree of cigarette smoke exposure and serum SP-A and SP-D levels. However, since the number of cigarettes smoked was learned based on the questionnaire, it was taken as more or less than 10 pcs/day. In our study, the serum SP-A level was statistically significantly higher in those who were in an environment where >10 pcs/day of cigarettes are smoked compared to those found in an environment where <10 pcs/day of cigarettes are smoked. While the SP-D level was also high, this was not statistically significant. There was also a correlation between the serum SP-A level and the urine cotinine level, which is an objective criterion for exposure to cigarette smoke. A study by Lomas *et al*. [14] found a positive correlation between cigarette consumption (packs/year) and serum SP-D levels. These results suggest that the serum SP-A level, an indicator of anti-inflammatory response, increases due to increased inflammation with exposure to cigarette smoke.

A questionnaire was carried out, and urine cotinine levels were measured to assess cigarette smoke exposure in our study. It is reported in various studies that information provided by parents on children's exposure to cigarette smoke does not correlate with the cotinine levels measured in children's urine; therefore, this information alone cannot be sufficient [24,25]. These results demonstrate the necessity of studying the cotinine level, which is an objective criterion, in addition to the information provided by families when determining exposure to cigarette smoke in children. In our study, the urine cotinine level the group exposed to cigarette smoke was significantly higher than that of the group not exposed to cigarette smoke.

Our study is the first study to evaluate serum SP-A and SP-D levels in children exposed to cigarette smoke as far as we know. This study shows that cigarette smoke affects the levels of SP-A and SP-D, which are among the most important substances of innate immunity in the lungs, and causes an increase in serum SP-A and SP-D levels as a response to the pulmonary inflammation that it causes. Cotinine, an objective indicator of exposure to cigarette smoke, is valuable in terms of ensuring the reliability of the results.

The limitation of this study was that while the serum SP-D level was high, the difference was not statistically significant and is related to the number of subjects. It will be useful to support this finding with other studies to be done. We think that re-examination of these children with long follow-up studies will be important for the evaluation of our findings. Cigarette smoke-free living areas must be provided for children. Prevention of exposure of children to this contaminant due to the adoption of more applicable legal and administrative measures, the development of advanced laboratory techniques to assist in the identification of problems and the public's awareness of the public's health.

CONCLUSION

In this study, SP-A and SP-D levels are higher than the children who are not exposed to environmental tobacco smoke may be interpreted as lung inflammation and lung injury and residues in response to inflammation in the lungs exposed to cigarette smoke.

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Funding: None; Conflict of Interest: None Stated.

How to cite this article: Huseyin G, Halil K, Zeyrek CD. Investigation of serum surfactant protein a and d levels in children exposed to cigarette smoke. Indian J Child Health. 2018; 5(10):607-610.

Doi: 10.32677/IJCH.2018.v05.i10.001