Exposure to house dust endotoxin and allergic sensitization in allergic and nonallergic children living in Adana, Turkey

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SUMMARY: Yılmaz M, Altıntaş DU, Bingöl-Karakoç G, İnal A, Kılıç M, Sütolukta Z, Güneşer-Kendirli S. Exposure to house dust endotoxin and allergic sensitization in allergic and nonallergic children living in Adana, Turkey. Turk J Pediatr 2009; 51: 225-231.

It has been suggested that exposure to elevated levels of endotoxin decreases the risk of allergic sensitization. The objective of our study was to analyze associations between house dust endotoxin levels and allergic sensitization in children.

One hundred children with self-reported allergic diseases and 100 healthy children were randomly selected from a list of a previous prevalence study in school children. These children attended the study center again to complete a detailed questionnaire and medical examination including skin prick test and pulmonary function test. Of these children, 65 had allergen sensitization. Parents of a total 100 children (50 allergic and 50 healthy) agreed to house dust sampling in their homes. Thirty-five allergic children had asthma and 25 had rhinitis. Thirteen allergic and 14 healthy children lived in rural areas. The endotoxin content was quantified using a chromogenic kinetic Limulus amoebocyte lysate test.

Endotoxin was at a detectable level in all dust samples. Endotoxin levels ranged from 0.05 to 309 EU/ml, with a geometric mean of 61.8 (confidence interval [CI] %) (50-73) EU/ml. There were no differences in house dust endotoxin levels between allergic and nonallergic children (p=0.153). On the whole, the mean level of endotoxin in rural homes was higher than that of urban homes, but this was not statistically significant (p=0.354). The highest endotoxin level was found in the homes of nonallergic children living in the rural areas and the lowest level in the homes of allergic children living in an urban area; however, this was not important statistically (p=0.320). Exposure to endotoxin was not associated with a risk of allergic sensitization (odds ratio [OR]=0.98; 95% CI: 0.91-1.05, p=0.609).

In conclusion, supposing that the current level of endotoxin may reflect that in the past, the levels of endotoxin in living room floor dust of homes of allergic and nonallergic children in our study population were not associated with allergic sensitization. Further studies are needed on this topic.

Key words: allergy, asthma, endotoxin, children.

The incidence and prevalence of asthma and allergic diseases have increased in developed countries in recent decades[1,2]. Although the reason for this rise is not exactly known, the hygiene hypothesis has gained strong support over the past years[3]. Exposure to microbial products in early life could be an underlying factor for this hypothesis, but the mechanisms underlying these protective effects are not known. The reduced microbial stimulation in early life may prevent maturation of Th-1 immunity leading to an allergen-specific Th-2 immune response following natural exposure to allergens[4-6]. This hypothesis has been supported

by epidemiological data, indicating an inverse association between frequency, pattern and type of infection and the prevalence of allergy and asthma[7,8]. However, some studies have found no effect of common specific and non-specific infectious diseases in early life[9,10].

Several recent reports have shown that living in a farm environment and keeping pets at home confer significant protection against the development of allergen sensitization and atopic diseases in children[11-14]. It was supposed that the exposure-elevated levels of endotoxin might be responsible for this effect. The hypothesis has been prompted by various studies that reported a reduced risk of atopy, hay fever and of asthma in farmers' children and adolescents[15-18]. In patients, however, a direct cause-effect relationship has not yet been demonstrated.

Endotoxin is a lipopolysaccharide that forms the outer layer of the cell membrane of all Gram-negative bacteria. Endotoxins are more or less ubiquitous in the environment and are present in normal indoor environments as constituents of house dust. Increased concentrations of house dust endotoxin have been reported for homes of farming families and households where children had regular contact with farm animals[14,18]. Furthermore, elevated endotoxin levels have been found in homes where animals were kept indoors[19,20]. They are known to have strong immune-stimulatory and proinflammatory properties. Endotoxins are believed to promote asthma by inducing airway inflammation and to have protective effects on atopy development[21]. In a study, an inverse relationship was found between the level of endotoxin exposure and the capacity of peripheral-blood leukocytes to produce interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-10, and IL-12 after stimulation with lipopolysaccharide, indicating a key regulator of TH1-type immune development[15]. There are some evidences that exposure to endotoxin levels present in the home environment can promote asthma[22,23]. The effect of endotoxin on immune response may change according to age, dose, and immune response at exposure and this may explain differences in these results[24].

Most of the studies investigating the relationship between endotoxin exposure and allergic sensitization come from western developed countries. However, little information is available from other areas. The objective of this study was to determine whether there is any relationship between house dust endotoxin levels and allergic sensitization in Turkish children.

Material and Methods

Subjects

The subjects included in this study were recruited from a previous prevalence study in Turkish school children in Adana, in southeast Turkey[25]. One hundred children with self-reported allergic diseases and 100 healthy children were randomly selected from the list of 3,470 subjects. The children who were selected attended the study center again to complete a detailed questionnaire and for a medical examination including a skin prick test and pulmonary function test. Of these children, 65 had allergen sensitization. Parents of a total of 100 children (50 allergic and 50 healthy) agreed to a house dust sampling in their homes. The study population consisted of 50 allergic children and 50 nonallergic children as a control group. Allergic children had at least one positivity in skin prick tests. Of allergic children, 35 had asthma and 25 had rhinitis. All children in the control group were had negative skin prick tests. Thirteen allergic and 14 healthy children lived in rural areas.

Asthma was defined as the occurrence of wheezing in the last 12 months and/or a doctor's diagnosis of asthma in that same period and/or current use of asthma medication. Allergic rhinitis was defined as the presence of a runny or blocked nose without having a cold or flu in the last 12 months, accompanied by itchy-watery eyes and/or some interference in daily activities due to the nose problem.

Skin Prick Testing

Skin prick tests were performed by the same investigator using standardized extracts of *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, grasses, cereals, tree I and II mixes, olive, molds, cat, dog, cockroach, hen's egg, and cow's milk (Allergopharma, Germany). Histamine dihydrochloride (10 mg/ml) and glycerol diluent were used as positive and negative controls, respectively. A wheal size greater than 3 mm was used as a positive result. The patients having at least one positivity in skin prick tests were considered as atopic.

Pulmonary Function Tests

Pulmonary function tests were measured with spirometry (ZAN 100 Spiromed, Germany). Forced expiratory volume in one second (FEV1) and peak expiratory flow (PEF) rate were measured and expressed as percentages of predicted mean values.

Questionnaire and Inspections of Homes

The International Study of Asthma and Allergies in Children questionnaire[26] was translated into Turkish and modified to obtain more information regarding keeping indoor and outdoor animal(s), house age, family size, duration living in the house, tobacco smoke exposure, and family history of allergic disease.

Collection, Storage, Extraction, and Endotoxin Analyses of House Dust

We visited all the houses in May 2006, and asked families not to clean the living room for at least 5 days prior to our visit. In each house, a dust sample was taken from the living room carpet according to a standardized protocol. All dust samples were taken using the same vacuum cleaner (1300 W, Philips, Turkey) with a dust-collection nozzle (Indoor Biotechnologies, UK) by vacuuming an area of 1 m^2 for 2 min. Samples were stored at –20°C until extraction.

Dust samples were extracted immediately before assay under sterile and pyrogen-free conditions with Limulus-Amebocyte-Lysate (LAL) reagent water + 0.05% Tween (v/v) for 2 h at room temperature with continuous shaking. Extraction ratios were between 1:10–1:100 (w/v) related to the amount of dust per sample. Suspensions were centrifuged at 1000 *g*. Endotoxin content of all dust samples was determined by a kinetic Limulus assay (Pyrochrome Limulus amebocyte lysate assay, Associates of Cape Cod; Falmouth, MA, USA) according to the manufacturer's instructions. Briefly, an aliquot of 50 µl suspension (1:1000) dilution was added to a microtiter plate (Pyroplate® 96-well, Associates of Cape Cod) and assayed with LAL. The optical density (OD) of each well was recorded at 405 nm every 30 s for 120 min during incubation at 37°C (Tecan, Switzerland). The endotoxin concentration was calculated by comparing the OD of samples to the OD standard calibration curve (0.05–2 EU/ml) included in the kit. The intra-assay variability (EU/mg dust) was less than 10%, whereas the inter-assay variability was lower than 20%. Resulting endotoxin levels were expressed as EU/m^2 (endotoxin concentration) and relative to weight of the sampled dust, as EU/mg dust (endotoxin load).

Ethical approval was obtained from Ethics Committee of Çukurova University. Parents of all children selected for the current study agreed to their child's participation and provided written informed consent.

Statistical Analysis

Because endotoxin levels in living room floor dust expressed as EU/m^2 were best described by a log normal distribution, statistical analysis was based on log-transformed data. Mean concentrations and load were expressed as geometric means (GM) with 95% confidence intervals (95% CI). Paired analyses were performed with Wilcoxon rank sum test and unpaired analyses with Mann-Whitney U test, and correlations with Spearman's rank order correlation coefficient test. The chi-square test was used for categorical variables. Multivariate logistic regression was used to adjust for potential confounders. Significance was set at a P value of.05. Statistical analysis was performed using SPSS 11.0.

Results

Description of Study Population

All children included in the study completed the sampling process of the study. The characteristics of the study population are presented in Table I. The number of boys was slightly higher than that of girls. There were 31 boys and 19 girls in the allergic group, and 30 boys and 20 girls in the control group. The two groups did not differ regarding their age and sex composition. Sensitization to house dust mite was the most common (44%), followed by pollen sensitization (39.3%), which was higher in rural areas. There were differences between asthmatic children and the control group according to their PEF and FEV1 values, with lower values in asthmatic cases than in the control group, and these differences were statistically significant ($p=0.025$ and $p=0.032$, respectively).

Table I. Characteristics of the Study Population

	Allergic group	Nonallergic group	P
Age of cases	11.5±.2.8	12.4±2.7	>0.05
Gender (F/M)	19/21	20/31	>0.05
House age (mean±SD)	16.7±11.1	12.9±7.9	>0.05
Number of family members (mean±SD)	6.2±2.2	5.6±1.6	>0.05
FEV1§	74±11.9	89±21.6	0.032
PEF§	71±9.1	86±11.6	0.025
Allergen sensitization			
House dust mite	27		
Pollen	19		
Food	8		
Animal	3		
Animal exposure			
Indoor*	16	17	>0.05
Outdoor**	13	17	>0.05

FEV1: Forced expiratory volume in 1 second. PEF: Peak expiratory flow.
§ Values of children with asthma.
*Dog, cat, birds, etc.
**Cow, goat, sheep, dog, cat, birds, etc.

Family Size, Age of Home, Exposure to Animal

Allergic and nonallergic children did not differ regarding their family size and house age. Indoor and outdoor animal exposure was much greater for children in rural areas than for those in urban areas (44/19). However, it did not differ between the allergic and nonallergic groups (29/34). Indoor animal exposure was more common in children with asthma than those without asthma (p=0.20, chi-square test), but this trend was not observed in children with allergic rhinitis (p=0.396).

Exposure to Endotoxin

Endotoxin was at a detectable level in all house dust samples. Table II shows the endotoxin levels in living room floor dust of both groups. Endotoxin levels ranged from 0.05 to 309 EU/ml, with a geometric mean of 61.8 (CI%) (50-73) EU/ml. There were no differences in house dust endotoxin levels between allergic and nonallergic children (p>0.05). The mean level of endotoxin in rural and urban homes was not statistically different in allergic and nonallergic groups (p>0.05).

Association Between Endotoxin and Allergic Symptoms and Diseases

Exposure to endotoxin was not associated with the risk of allergic sensitization (OR=0.98 95% CI: 0.91-1.05, p>0.05). Endotoxin levels were similar in children with asthma and those with rhinitis (p>0.05), neither of whom differed from the nonallergic children in this respect (p>0.05 and p>0.05, respectively). In addition, endotoxin exposure in children who had had wheezing in the last 12 months (mean 86.3 EU/ml) did not differ from those with no

Table II. Endotoxin Levels in Living Room Floor Dust in Both Groups

	All Mean (95% CI) Range	Urban area Mean (95% CI) Range	Rural area Mean (95% CI) Range	p
Allergic	58.4 (42.0-74.8) 0.5-309	54.6 (38.8-70.5) 0.5-239	68.9 (19.5-118.4) 3.0-309	>0.05
Nonallergic	62.7 (47.6-76.8) 0.5-288	65.7 (51.5-79.9) 0.5-219	72.5 (28.4-116.7) 3-288	>0.05
P	>0.05	>0.05	>0.05	

CI: Confidence interval.

wheezing in the last 12 months (mean 77.9 EU/ml) and from healthy children ($p>0.05$). The levels of endotoxin in allergic children were not correlated with PEF and FEV1 ($r=0.041$, $p>0.05$ and $r=0.017$, $p>0.05$, respectively). No association was found between endotoxin levels and family size and house age ($r=0.120$ and $r=0.168$, respectively). Animal exposure, tobacco smoke exposure, and family history of allergic disease were not associated with the level of endotoxin ($p>0.05$).

We also analyzed associations between allergic sensitization and endotoxin levels expressed as EU/g dust instead of EU/m^2 (data not shown). The endotoxin concentration was highly correlated with endotoxin load ($r=0.886$).

Discussion

In this study, we found that house dust endotoxin concentration in allergic and nonallergic children was similar. In some studies of children, as in our study, no consistent association was found between endotoxin level and the development of allergic diseases[27,28]. Some studies from developed and developing countries showed an inverse relationship between exposure to house endotoxin and the frequency of atopic asthma, hay fever, and allergic sensitization in children and in adults[13-18,29]. However, some studies showed that increasing rather than decreasing endotoxin exposure is associated with an increased risk of asthma and allergic sensitization[22,23,30,31].

We did not observe an association with current wheezing and abnormal pulmonary function test in our study population. Several studies showed that endotoxin in house dust is associated with exacerbations of pre-existing asthma in children and adults[32,33]. Some studies also showed that domestic endotoxin levels in asthmatic children were significantly correlated with a decrease in FEV1 and an increase in symptoms and daily need for oral and inhaled asthma medication[22,23].

The immune stimulatory potency of endotoxin may depend on timing of exposure relative to disease development (e.g., early life exposure), dose and frequency of stimulus, coexposure, and immune responsiveness to endotoxin[24]. This can explain the differences in the results of the various studies. Exposure to bacterial endotoxin in neonatal life has been proposed to play a role[17]. Tulic et al.[21] showed, in an animal model, that exposure to bacterial lipopolysaccharide immediately before or shortly after primary allergen sensitization inhibits the increase of allergen-specific IgE levels. Riedler et al.[14] demonstrated that exposure to stables in or before the first year of life is crucial for the protective effect. However, there is some evidence that exposure to bacterial endotoxin may induce respiratory symptoms in children. Higher house dust endotoxin levels are also associated with more wheezing symptoms in the first year of life[33]. Possible explanations for this association of endotoxin exposure with increased asthma symptoms at any age include an adjuvant-like effect of endotoxin exposure on airways inflammation.

Genetic variations and immune stimulatory microbial components in dust might be one reason for the discrepancy in results between this study and other studies from developed countries. The role of these components has to be investigated in the framework of cohort studies. It stands to reason that genetic variation in the immune response to endotoxin influences either the benefit or harm of endotoxin exposure. A polymorphism in CD14 or TLR-2 was associated with the alterations in immune response to endotoxin[34-37].

Unlike other studies, we found no association between the presence of pets, family size and house age and endotoxin level of house dust[38-41]. Several factors are known to affect endotoxin levels. The presence of pets has been a significant determinant of endotoxin levels in many earlier studies[38-40]. This discrepancy between the results of our study and those of other countries may be due to the differences in lifestyle and housing characteristics. The lifestyle and house conditions of Turkish families differ in many respects from those in other countries. For example, Turkish families have larger family size and fewer pets, and they frequently heat their houses with wood or coal. There are also differences with respect to personal hygiene measures and traditional dietary habits.

There are some limitations of the present study. First, the small size of the population may lead to low statistical power. Although 140 children were initially enrolled, we were able to take samples from the houses of only

100 children. Second, exposure to house dust endotoxin was measured when the children were between 7 and 14 years of age. About half of the children in our study population were still living at their birth address. Thus, no information on early exposure to house dust endotoxin is available. There is scarce knowledge about whether or not a single endotoxin measurement represents exposure for a longer time period. Endotoxin measurements in dust have been reported to show little variation over time. Environmental endotoxin levels are therefore likely to reflect longer-term exposure to microbial compounds[15,16,42,43]. We think that the current level of endotoxin may reflect that of the past. Nevertheless, further studies and data are needed.

In conclusion, the levels of endotoxin in living room floor dust of allergic and nonallergic children in our study population were not associated with allergic sensitization. Further studies and data are needed to clarify the mechanism of allergic sensitization and allergic diseases considering environmental factors and genetic factors.

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