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Potential association between allergic diseases and pertussis infection in schoolchildren: Results of two cross-sectional studies seven years apart

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KEYWORDS

Allergic disease;
Anti-pertussis IgG;
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Children;
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Abstract

Background: In this study, we aimed to investigate the relationship between pertussis infections and allergic diseases in two cross-sectional questionnaire-based surveys carried out in 1997 and 2004. We also measured serum level of antibody to *B. pertussis*.

Material and Methods: Two cross-sectional, questionnaire-based surveys were carried out in 1997 (n = 3164) and 2004 (n = 3728). 361 cases and 465 controls were recruited from both surveys. The skin tests were performed using standardised extracts. The level of pertussis specific IgG was measured in 136 allergic and 168 non-allergic children.

Results: We found that allergic diseases prevalence was significantly higher in the children suffering from pertussis infections (22.3% first and 8.8% second survey) compared to children who did not suffer from pertussis infections (6.6% first and 4.5% second survey) (p = 0.001 and p = 0.035, respectively). Asthma prevalence was also significantly higher in children suffering from pertussis infection (37.6% first and 26.2% second survey) compared to children who did not suffer from pertussis (7.4% first and 5.0% second survey) (p = 0.001 and p = 0.001, respectively). However, the mean serum levels of anti-pertussis IgG were similar in allergic and non-allergic groups (p > 0.05).

Conclusion: Although pertussis antibody levels in atopic and non-atopic children were similar to each other, pertussis infection still seemed to have a significant effect on the development of atopic diseases.

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Introduction

The incidence and prevalence of asthma and allergic diseases have significantly increased in many countries in recent decades^{1,2}. Although the reasons for such a rise are not exactly known, the hygiene hypothesis has gained strong

support over the past years³. Exposure to microbial products in early life could be an underlying factor for this hypothesis, but the mechanisms underlying these protective effects are not exactly known. The reduced microbial stimulation in early life may prevent maturation of Th1 immunity leading to an allergen-specific Th2 immune response following natu-

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ral exposure to allergens^{4,6}. Several studies suggested that there was an inverse association between the incidence of allergic diseases and infections. It has been suggested that a powerful Th1 inducing infection, during or prior to allergic sensitisation, should decrease or protect against the development of allergic diseases^{7,8}.

Bordetella pertussis is a gram-negative bacterium which causes pertussis or whooping cough. It has previously been reported that *B. pertussis* infection stimulates Th1 type immune responses⁹. Recovery from infection is associated with the development of *B. pertussis*-specific Th1 cells in humans and mice¹⁰. Evidence for an association between pertussis and atopy is conflicting. In children with pertussis infection, IgE to pertussis toxin and common allergens, as well as increased total serum IgE levels, can be detected¹¹. However, a study indicated that pertussis in childhood did not predispose to subsequent asthma¹². The possible influence of *B. pertussis* vaccine on the development of allergy has been widely investigated. While previous reports postulated that pertussis vaccine constitutes a risk for the development of allergic diseases^{13,14}, to the best of our knowledge this could not yet be confirmed by further recent studies^{15,16}.

In the present study, we investigated the relationship between physician-diagnosed pertussis, and compared the results of two cross-sectional data from ISAAC written questionnaire-surveys conducted in 1997 and 2004. We also examined whether serum antibody levels to *B. pertussis* were different in cases of both surveys. Since prevalence of asthma and allergic diseases were previously dealt with in other studies, our main focus with this study was primarily on prevalence of pertussis infections.

Material and Methods

Two cross-sectional, questionnaire-based surveys were carried out in 1997 (n = 3164) and 2004 (n = 3728) in Adana, Turkey^{17,18}. The questionnaire, identical in both surveys, was completed by parents of children younger than 12 years old, and by the students themselves if older than 12. Both surveys were conducted over the same months of March-June. The cluster sampling method was similar in both surveys. Standard ISAAC core questions for wheezing, asthma, rhinitis and eczema were used. The questionnaire also included questions on age, gender, socioeconomic status, type of residence, environmental factors such as dampness in the house, smoking exposure, and current pet ownership. The following questions were also added to the questionnaire to identify whooping cough: "Have you/has your child ever had whooping cough?" "Have you/has your child ever had been diagnosed whooping cough by doctor?"

Asthma was defined as the occurrence of wheezing and/or a doctor's diagnosis of asthma and/or use of asthma medication. Allergic rhinitis was defined as the presence of a runny or blocked nose without having a cold or flu, accompanied by itchy-watery eyes and/or some interference of this nose problem with daily activities. Eczema was defined as itchy skin rash that has been recurring for at least 6 months and affecting particular skin sites (i.e. folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes).

Based on these criteria 361 cases with a mean age of 10.3 ± 1.4 years (155 male, 206 female) and 465 controls with a mean age of 10.9 ± 1.3 years (210 male, 255 female) were recruited from a random list of both surveys. These cases and controls again attended the study centre for a detailed questionnaire, skin prick test, and blood sampling. Blood samples were allowed to clot at room temperature for one hour, then cooled in a refrigerator for 1-2 hours before being centrifuged. Sera were stored at -80°C until the serologic analysis was carried out.

The skin tests were performed by the same investigator using standardised extracts of *Dermatophagoides pteronyssinus*, *Dermatophagoides farinea*, grasses, cereals, tree I and II mixes, olive, moulds, cat, dog, cockroach, hen's egg, cow's milk, and cacao (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. Patients with at least one positivity in skin prick tests were considered atopic.

The level of pertussis specific IgG was measured in 136 allergic (56 male, 80 female; mean age 10.1 ± 0.8 years) and 168 non-allergic children (72 male, 96 female; mean age 10.0 ± 0.6 years). Of the children who took part in the survey in 1997, 89 were allergic, and 106 were non-allergic. In the survey of 2004, 47 were allergic and 62 were non-allergic. Of allergic children, 95 had asthma, 74 had rhinitis, and 15 eczema. All children had received a whole cell pertussis vaccine combined with diphtheria and tetanus toxoid at 2, 4 and 6 months of age, and a booster dose at 18 months of age.

Pertussis antibody was determined by an enzyme linked-immunosorbent assay (ELISA) using a commercial kit (DRG Diagnostic, Germany) which detects antibodies against pertussis toxin and other pertussis antigens. The kit was used according to the manufacturer's instructions. The optical density (OD) was recorded with a 450 nm length wave reader (Medispec Esr-200). Results were measured as OD and expressed as arbitrary unit (U.) according to the instructions. Samples were considered positive if the absorbance was 10% above the cut-off value.

The study protocol was approved by the Ethic Committee of University of Cukurova. Written informed consent was obtained from the parents of all the participating children.

The statistical analyses were made using SPSS-X version 11.0 for Windows and Epi-info6.0. Antibody levels were expressed as median and interquartiles. As data was not normally distributed, the Man-Whitney U-test was used to compare the differences between groups. The Fisher exact test was applied when assessing the differences in positive and negative pertussis children.

Results

A total of 429 (13.6%) in the first, and 210 (6.3%) in the second survey had suffered from *B. pertussis* infection. We found that pertussis infections had significant effect on the prevalence of atopic diseases such as asthma and allergic rhinitis in both surveys. In these two surveys, allergic diseases prevalence was significantly higher in the children suffering from pertussis infections (22.3% first and 8.8% second survey) compared to children who did not suffer from per-

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