Allergol Immunopathol (Madr). 2013;xxx(xx):xxx-xxx

Allergologia et immunopathologia

www.elsevier.es/ai

ORIGINAL ARTICLE

Elevated level of serum osteopontin in school-age children with asthma

A.Z. Akelma[a,*], M.N. Cizmeci[b], M.K. Kanburoglu[b], D. Bozkaya[b], F. Catal[c], E. Mete[a], I. Kutukoglu[b], M. Namuslu[d]

[a] Department of Pediatric Allergy, Fatih University Medical School, Ankara, Turkey
[b] Department of Pediatrics, Fatih University Medical School, Ankara, Turkey
[c] Department of Pediatric Allergy, Inonu University Medical School, Malatya, Turkey
[d] Department of Biochemistry, Fatih University Medical School, Ankara, Turkey

Received 9 October 2012; accepted 2 January 2013

KEYWORDS
Osteopontin;
Childhood asthma;
Preschool children;
Persistent wheezing

Abstract
Background: The role of osteopontin (OPN) has not been elucidated in childhood asthma.
Objective: Our purpose was to investigate whether OPN levels change due to allergic inflammation in pre-school and school-age children.
Methods: In this prospective, cross-sectional study, 42 healthy children and a total of 51 children with asthma were recruited. OPN levels and its association with clinical and laboratory parameters were investigated in the study population. The asthma group were divided into two groups with respect to age, ≤5-years ($n = 23$) and >5-years ($n = 28$), and labelled Asthma Group 1 and Asthma Group 2, respectively. OPN levels were compared between subgroups.
Results: Serum OPN levels were significantly higher in the asthma group when compared to the control group ($p = 0.004$). OPN levels were similar in Asthma Group 1 and control groups, whereas it was found to be higher in Asthma Group 2 ($p > 0.025$, $p = 0.001$, respectively). In the >5-years age asthmatic group, OPN levels of the patients with allergic rhinitis ($n = 15$) were higher than those of the patients ($n = 13$) without allergic rhinitis ($p = 0.021$).
Conclusion: The study underscores the relationship between childhood asthma and OPN as the first study in the literature. In this study we found that OPN, which plays a role in Th2 mediated inflammation, may also play a role in childhood asthma. The fact that OPN levels do not increase in preschool-age children with asthma might be due to the transient wheezing in this group.

Introduction

Asthma is a chronic inflammatory disease of the airways characterised by Th2-mediated inflammation and bronchial hyperreactivity. It typically presents with wheezing and dyspnoea in children. In preschool age, wheezing and asthma

* Corresponding author.
E-mail address: akelma@gmail.com (A.Z. Akelma).

0301-0546/$ - see front matter © 2012 SEICAP. Published by Elsevier España, S.L. All rights reserved.
http://dx.doi.org/10.1016/j.aller.2013.01.009

are heterogeneous disorders with many phenotypic and variable expressions. Although almost half of the children are reported to have wheezing in the first six years of life, only 40% of these toddlers will experience persistent wheezing symptoms in later childhood.[1] Asthma in children older than school age shows typical features of Th2-mediated inflammation. However, it is not the same for preschool children with asthma. The progression of clinical features of preschool and school-age children with asthma might not be the same due to possible pathophysiological differences between these two groups. However, there is not a definitive biomarker to identify children with high-risk phenotypes who will go on to have persistent asthma.

Osteopontin (OPN) is identified in many cell types in the immune system. It has been shown to be produced especially by T-cells, B-cells, macrophages, neutrophils, eosinophils, natural killer cells, CD11c-positive dendritic cells (DCs) and bronchial epithelium. OPN is a protein expressed during the inflammatory processes related to Th2 lymphocyte activity.[2] It was demonstrated in previous studies that OPN plays role in asthma,[3–7] allergic rhinitis,[8] allergic conjunctivitis[9] and response to venom immunotherapy.[10] However, the role of OPN in paediatric asthma has not been studied.

In this study, we aimed to investigate whether OPN levels were affected by allergic inflammation in preschool and school-age children. The relationship between OPN and clinical and laboratory parameters along with inflammatory cytokines for pre-school and school-age children with asthma was examined.

Materials and methods

Study subjects

This prospective, cross-sectional study was conducted with 93 children, 51 of whom suffered from asthma and 42 of whom were healthy controls, presenting to the Fatih University paediatric allergy and well-child outpatient clinics between March 2011 and April 2012. The asthma group included children of ages $\leq$5 years, with at least four wheezing attacks during the previous year, and ages >5 years who were diagnosed clinically and functionally according to the GINA criteria (Asthma Group 1 and Asthma Group 2, respectively). Treatment of the children with asthma was arranged according to the updated GINA guidelines.[11] Children of similar age and gender with no history of allergic disease or wheezing were selected as the control group. The control group was divided into two groups according to their ages, $\leq$5 and >5 years (Control Group 1 and Control Group 2, respectively). Those with chronic diseases (e.g. malnutrition, anatomic malformation of the respiratory system, chronic lung disease, heart disease, gastro-oesophageal reflux disease, cystic fibrosis) and those with a history of chronic drug use (e.g. antiepileptics, immune suppressives) were excluded from the study.

Venous blood samples were collected into Vacuette tubes (Greiner Bio-One, Monroe, NC, USA) and centrifuged at 3000 *g* for 15 min at 4 °C. Serum samples were stored at −80 °C for no more than six months. Levels of IL-4, IL-6 and IL-10 were measured by the EASIA (Enzyme Amplified Sensitivity Immunoassay) method using a ELX-800 system (DIAsource, Nivelles, Belgium). Levels of IL-13, IL-17, transforming growth factor beta (TGF-β) and osteopontin were measured by the ELISA (Enzyme Linked Immunosorbent Assay) method using a ELX-800 system (RayBiotech, Norcross, GA, USA). Levels of high sensitive C reactive protein (hsCRP) were measured by turbidimetric assay method using a Roche P 800 modular system (Hitachi, Tokyo, Japan). Levels of eosinophil cationic protein (ECP) were measured by the chemiluminescent assay method using an Immulite 2000 systems (Simens, Llanberis, Gwynedd, UK). The eosinophil counts were measured by LH-780 system (Beckman Coulter, Mervue, Galway, Ireland). Levels of total serum IgE were measured by the ECLIA (Electrochemiluminescence) method using an ELX-800 system.

Atopy in the asthma group was investigated using a skin prick test (SPT) and specific IgE (sIgE) measurements. A test was considered positive if the SPT results demonstrated a wheal with a mean diameter of at least 3 mm greater than that of a saline control. Each child was tested with a core battery of allergens (e.g. dust mite, cockroach, cat, dog, mould, grass, tree, weed, milk, egg, and peanut) and a clinic-specific battery of locally relevant allergens (ALK Abelló, Hørsholm, Denmark). Spirometry (Vmax encore; VIASYS Healthcare Inc., Conshohocken, PA, USA) and bronchodilator reversibility were defined as greater than a 12% or 200 ml change from baseline FEV_1. These parameters were measured according to the GINA criteria.[11]

The study was initiated upon approval by the Local Ethics Committee of Fatih University in accordance with the Helsinki Declaration. The written informed consent of the parent(s) of each subject was also obtained before the study.

Statistical analysis

Data analysis was performed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). Whether the continuous variables were normally distributed was determined by using Shapiro–Wilk test. Homogeneity of variance was evaluated by the Levene test. Data were shown as mean ± SD or median (min–max), where applicable. When there were two independent groups, medians were compared using the Mann–Whitney *U*-test. Differences between the medians of more than two groups were evaluated by using the Kruskal–Wallis test. When the *p*-value from the Kruskal–Wallis tests were found to be statistically significant, Conover's non-parametric multiple comparison test was used to identify which group differed from the other. The mean ages between groups were compared using the *t* test. Nominal data were analysed using Pearson's Chi-Square or Fisher's exact tests, where appropriate. Degrees of association between continuous variables were calculated by the Spearman's correlation coefficient. A *p* value of less than 0.05 was considered statistically significant. For all possible multiple comparisons, the Bonferroni Correction was applied for controlling Type I error.

Power analysis

In $\leq$5-years age group, we planned to enrol 23 patients with asthma and 21 control subjects. The mean values and standard deviation of OPN were 6.16 ± 1.52. We calculated

Please cite this article in press as: Akelma AZ, et al. Elevated level of serum osteopontin in school-age children with asthma. Allergol Immunopathol (Madr). 2013. http://dx.doi.org/10.1016/j.aller.2013.01.009