Original article

Salivary cortisol levels and allergy in children: The ALADDIN birth cohort

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Background: Pre- and postnatal stress have been related to allergy in children, but evidence from prospective studies is limited. Several environmental factors can influence the salivary cortisol level, which is used as a measure of activity of the hypothalamic-pituitary-adrenal axis.

Objective: The aim of this study was to assess the association between salivary cortisol levels at 6 months of age and allergic manifestations during the first 2 years of life.

Methods: Salivary samples for the analysis of cortisol level were collected at 6 months of age on 3 occasions during 1 day from 203 children. Blood samples were collected at 6, 12, and 24 months of age for analyses of specific IgE. Information on allergy-related symptoms was obtained by repeated examinations of the children. Generalized estimating equation statistics were used to calculate the overall risk for outcome measures.

Results: The adjusted odds ratio for the relationship between morning cortisol level and IgE sensitization was 1.60 (95% CI, 1.22-2.10, $P = .001$) and for eczema it was 1.28 (95% CI, 1.03-1.59, $P = .026$). The odds ratio for afternoon cortisol level in relation to sensitization and eczema was 1.56 (95% CI, 1.26-1.94, $P < .001$) and 1.33 (95% CI, 1.12-1.58, $P = .001$), respectively, and for evening cortisol level it was 1.49 (95% CI, 1.22-1.83, $P < .001$) and 1.37 (95% CI, 1.18-1.59, $P < .001$). Salivary cortisol level in the evening was associated with food allergy.

Conclusion: The association between salivary cortisol levels in infancy and allergic sensitization and allergic symptoms suggests a role of an altered hypothalamic-pituitary-adrenal axis in the etiological process of allergies. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: *Allergy, sensitization, salivary cortisol, children, stress*

Several environmental factors have been associated with the rising prevalence of IgE-mediated allergic diseases in children during the past decades.[1-3] Besides focus on the types of factors influencing the occurrence, the timing of exposure has been discussed, especially the pre- or early postnatal period.[2,4] A relationship between psychosocial factors and allergic diseases is documented.[5] Levels of salivary cortisol have been used and validated as a measure of activity of the hypothalamic-pituitary-adrenal (HPA) axis.[6] An altered function of the HPA axis has been observed after several types of psychosocial exposures and may also influence immunologic processes.[7] Maternal prenatal stress measured by questionnaires and different lifestyle factors such as household income have been related to immunologic changes in cord blood and to later development of allergic disease in children.[8-11] Furthermore, postnatal exposure to stress has been associated with the etiology of asthma.[12,13] Increased responsiveness of the HPA axis to stress has been seen in healthy infants with atopic predisposition and in infants with exposure to risk factors for allergic disease.[14,15] Children with established allergic disease differ in cortisol response to stress and have altered basal cortisol levels compared with healthy children.[16,17] We have previously shown that school children of families with an anthroposophic lifestyle seem to have a lower prevalence of allergic diseases[18-20] and recently that infants in these families have low levels of salivary cortisol and of allergic sensitization.[21,22] The aim of this study was to investigate whether salivary cortisol levels early in life are associated with the development of allergic sensitization and symptoms during early childhood.

METHODS

Population

This study is based on Assessment of Lifestyle and Allergic Diseases During Infancy (ALADDIN), a prospective birth cohort study that focuses on the impact of lifestyle and environmental factors during pregnancy and childhood on the development of allergic disease. The study design has

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Abbreviations used
ALADDIN: Assessment of Lifestyle and Allergic Disease During Infancy
HPA: Hypothalamic-pituitary-adrenal
OR: Odds ratio

previously been described in more detail.[22] In brief, 330 families were enrolled from September 2004 until November 2007 in gestational week 28 to 32 and followed prospectively until the age of 24 months of the child. In order to include families of diverse lifestyles, they were recruited from anthroposophic and conventional maternal-child health centers in urban and rural areas of Stockholm County. Families with infants born before gestational week 36 or families with miscarriages were excluded. The study was approved by local ethics committee, and written informed consent was obtained from all families.

Procedures

Salivary samples were obtained at home from the infant during 1 day at the age of 6 months in the morning, afternoon, and evening. The procedure of saliva collection has previously been described.[21] In brief, the morning sampling occasion was defined as "a quarter of an hour after awakening and before first meal"; afternoon as "after midday sleep" (or alternatively, if the child did not sleep, "1 hour after midday meal"); and evening as "before going to bed." The parents also answered a short questionnaire regarding any unusual events or health problems of the child during the day of sampling. The saliva samples were collected by sterile rolls (braided cotton dental rolls; Richmond Dental, Charlotte, NC), which the parents were instructed to keep in the mouth of the child for about 1 to 3 minutes until soaked with saliva. The samples were centrifuged and stored at −80°C until analyzed, according to the manufacturer's instructions, by using the Spectria Cortisol RIA (125l) kit from Orion Diagnostica, Espoo, Finland.[23] Samples from the same infant were analyzed in the same assay.

Blood samples were obtained from the parents at inclusion in the study and from the children at 6, 12, and 24 months of age. The samples were collected in heparin tubes, and plasma was stored at −20°C. Parental IgE sensitization was defined by using ImmunoCAP Phadiatop (Phadia AB, Uppsala, Sweden) containing a mix of 11 common inhalant allergens. Blood from the children was analyzed by using ImmunoCAP (Phadia AB) for IgE to 7 allergens (hen's egg, cow's milk, peanut, cat, dog, birch, and timothy grass). A study subject was classified as sensitized if IgE levels were ≥0.35 kU_A/L for the parents in Phadiatop and for the children in at least 1 of the 7 allergens.

The children were followed prospectively from birth until the age of 2 years and were examined by 1 of the study doctors at 2, 6, 12, 18, and 24 months of age, with a particular focus on the presence of allergy-related disease. The diagnosis of eczema was based on the criteria of the UK Working Party's refinement of the Hanifin and Rajka criteria.[24] *Food allergy* was defined as an acute onset of symptoms such as skin reactions, wheezing, vomiting, or diarrhea on more than 1 occasion after ingestion or contact with a particular type of food. *Recurrent wheeze* was defined as 3 or more episodes of wheeze since the last examination.

Statistical analyses

Statistical analyses were conducted by using PASW 18.0 software (SPSS, Chicago, Ill). Since salivary cortisol levels showed a skewed distribution, logarithmic transformation was used in the statistical computations. The log-transformed saliva cortisol concentration was approximately normally distributed. The degree of adherence to an anthroposophic lifestyle was categorized into 3 lifestyle groups as "anthroposophic," "partly anthroposophic," and "nonanthroposophic" on the basis of the choice of the maternal-child health center and answers to 3 questions on anthroposophic lifestyle, which are described in detail elsewhere.[22] General estimated equations were used to assess the association between salivary cortisol level in the morning, afternoon, and evening and sensitization, eczema, food allergy, and recurrent wheeze. The general estimated equation model calculates the average risk by taking into account the correlation within individuals as well as unequally spaced missing observations. The models were adjusted for sex of the child, lifestyle group, parental sensitization, mother smoking during pregnancy, number of siblings or other children living with the family, exclusive breast-feeding at age 6 months, family living on a farm with animals during pregnancy, and parental education. Interaction between lifestyle group and salivary cortisol level was analyzed. *P* values less than .05 were regarded as statistically significant.

TABLE I. Demographic characteristics of the study population

	n/N	Percent
Sex (females)	101/198	51
Mother education: high school or higher	171/198	86.4
Father education: high school or higher	165/196	84.2
Maternal sensitization	59/198	30.0
Paternal sensitization	80/198	40.4
Exclusive breast-feeding at 6 mo of age	19/198	21.0
Family living on a farm with animals during pregnancy	22/198	11.0
Mother smoking during pregnancy	12/198	6.1
Lifestyle group		
Anthroposophic	46/198	23.2
Partly anthroposophic	76/198	38.4
Nonanthroposophic	76/198	38.4

RESULTS

At 6 months of age of the child, 305 families were included in the ALADDIN study. During the follow-up, 5 of 305 (1.6%) families dropped out from the study: 1 because of moving, 1 because of psychosocial factors, 1 because of disease other than allergy, and 2 because of unknown reasons. When the child was 6 months old, 203 families collected salivary samples of the child and of them 198 collected at least 1 blood sample (at 6, 12, and/or 24 months of age).

The distribution of the adjustment variables is shown in Table I. The parents had a prevalence of allergic sensitization comparable to that of the general population. None of the families reported treatment of the child with inhalation or oral corticosteroids at age 6 months when the salivary sample was collected. The mean number of siblings or other children living with the family was 0.83.

The mean (geometric means with 95% CIs) salivary cortisol levels at age 6 months varied with a diurnal rhythm: 11.7 nmol/L (95% CI, 10.1-13.5) in the morning, 5.1 nmol/L (95% CI, 4.3-6.0) in the afternoon, and 2.9 nmol/L (95% CI, 2.4-3.5) in the evening. The mean sampling times were 7:42 (±1:04 hours) in the morning, 15:06 (±1:54 hours) in the afternoon, and 19:54 (±1:20 hours) in the evening.

The prevalence of allergic sensitization increased during the follow-up from 11% at 6 months of age to 18.8% at 24 months of age (Table II). At age 2 years, 9.9% of the children had eczema, 4.2% reported food allergy, and 6.0% reported recurrent wheeze.

Salivary cortisol levels on all sampling occasions were related to the prevalence of sensitization and eczema during the first 2 years of life, with increasing levels of cortisol leading to higher prevalence of sensitization and eczema (Fig 1). The overall adjusted odds ratio (OR) for the association between the morning saliva cortisol level and sensitization during the first 2 years was 1.60 (95% CI, 1.22-2.10, $P = .001$) and the corresponding

TABLE II. Prevalence of the outcome measures: sensitization, eczema, food allergy, and recurrent wheeze at time points when assessed

	Sensitization		Eczema		Food allergy		Recurrent wheeze	
Age	n/N	Percent	n/N	Percent	n/N	Percent	n/N	Percent
2 mo	—	—	10/296	3.4	—	—	—	—
6 mo	24/218	11.0	22/287	7.7	—	—	—	—
12 mo	39/220	17.7	26/283	9.2	16/287	5.6	8/285	2.8
18 mo	—	—	24/272	8.8	15/274	5.5	21/272	7.7
24 mo	45/240	18.8	28/283	9.9	12/283	4.2	17/284	6.0

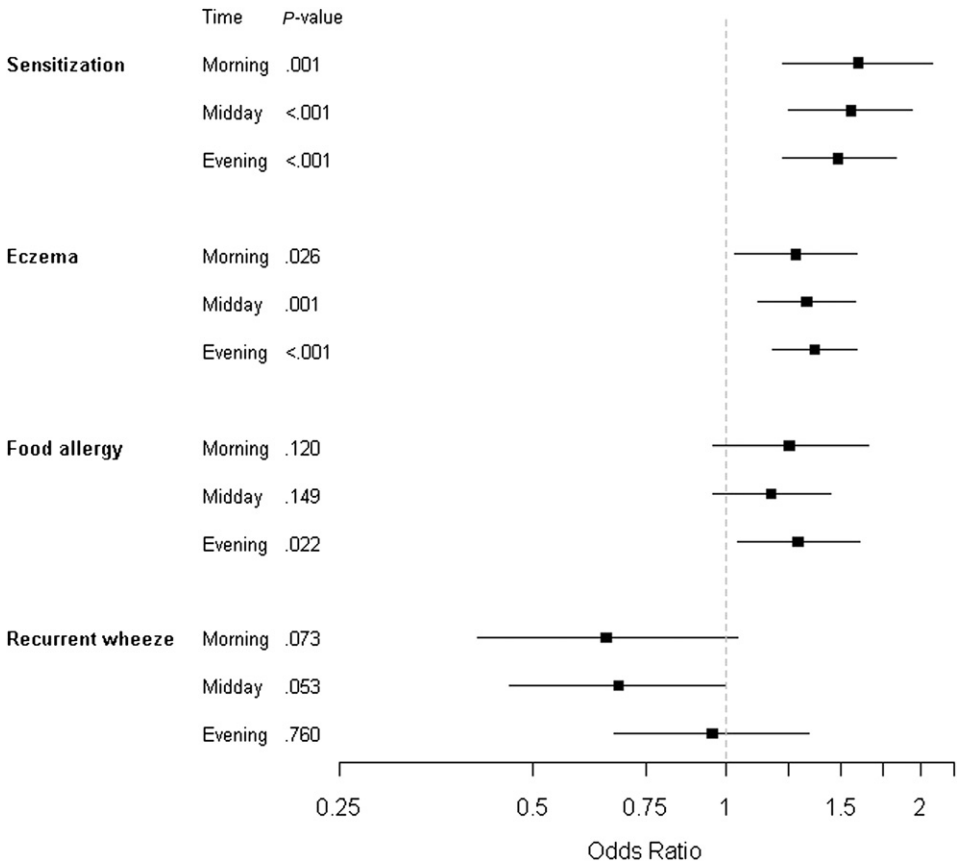

FIG 1. OR and 95% CI for allergic sensitization and allergy-related disease during the first 2 years in relation to the saliva cortisol level in 6-month-olds at different time points of the same day.

adjusted OR for eczema was 1.28 (95% CI, 1.03-1.59, $P = .026$). The OR for sensitization and eczema in relation to afternoon levels of salivary cortisol was 1.56 (95% CI, 1.26-1.94, $P < .001$) and 1.32 (95% CI, 1.12-1.58, $P = .001$), respectively, and for the evening levels 1.49 (95% CI, 1.22-1.83, $P < .001$) and 1.37 (95% CI, 1.18-1.59, $P < .001$), respectively. Salivary cortisol level in the evening was also significantly associated with subsequently reported food allergy, with overall OR being 1.29 (95% CI, 1.04-1.61, $P < .022$). Salivary cortisol level was, however, not related to recurrent wheeze at any time point, but there was a tendency to decreased prevalence with higher levels of cortisol. There was no significant interaction between salivary cortisol level and lifestyle group on the occurrence of allergic diseases.

To further analyze the impact of salivary cortisol levels at age 6 months on only the later development of sensitization and eczema, the analyses were restricted to healthy children (not sensitized or without eczema) at 6 months of age. Among children without sensitization at age 6 months, there was a close to significant association between salivary cortisol level in the afternoon at age 6 months and sensitization from 12 to 24 months of age, with adjusted OR of 1.43 (95% CI, 1.00-2.06, $P = .051$). The corresponding ORs for the morning and evening levels were 1.30 (95% CI, 0.90-1.87, $P = .16$) and 1.29 (95% CI, 0.95-1.75, $P = .11$), respectively. On the other hand, salivary cortisol level at age 6 months following this exclusion was not related to the development of eczema between 12 and 24 months of age.

DISCUSSION

Our novel findings of associations between salivary cortisol level in infancy and allergic sensitization as well as allergic symptoms during the first 2 years of life suggest a role of the HPA

axis in the early development of allergy in children. An association between pre- and postnatal stress and subsequent development of allergic diseases has previously been indicated.[8-11] Furthermore, it has been shown that infants predisposed to allergic disease have higher levels of cortisol prior to the onset of disease.[14,15] However, this is, to our knowledge, the first study showing that high levels of cortisol could precede the development of allergic sensitization and allergic disease. Thus, the blunted cortisol response to stress (hyporeactivity) and the lower levels of cortisol found in children with chronic manifestations of allergy may be reversed (hyperreactivity) before or at the onset of allergic disease.[16,17,25]

Our findings of elevated levels of salivary cortisol accompanied by higher prevalence of allergic sensitization, eczema, and food allergy could suggest a causal association, but this has to be interpreted with care. A reverse causation could exist, meaning that sensitization and allergic symptoms evoke increased levels of cortisol. However, the outcomes in our analyses were studied with a longitudinal design and the association between afternoon cortisol levels in healthy children at 6 months of age and later sensitization indicates that the levels are related to subsequent development of sensitization and allergic symptoms.

There was no correlation between cortisol level and recurrent wheeze. Wheeze is a heterogeneous manifestation and not considered to be linked to IgE early in life[26] and thus may be expressed via pathways other than the HPA axis. The correlation between cortisol level and food allergies was significant only for the evening level as compared with that between cortisol level and eczema and sensitization, which was correlated on all 3 occasions. The explanation for this is not clear, but it may reflect the more chronic character of the symptom eczema. In addition, food allergy was assessed by the parental medical history of the child, thus suffering from less validity and precision than sensitization data and eczema examined by a doctor.

The strengths of our study include the prospective design with families recruited already during pregnancy and data collected longitudinally. The variables cortisol level and sensitization were based on objective measurements independent of each other. The cortisol values were comparable to proposed reference values for infants.[27] A collection of salivary samples for analyses of cortisol was scheduled to not interfere with the health care visit and blood drawing. The study doctor was blinded to salivary cortisol levels when doing clinical assessments. None of the families received any results of the blood samples or cortisol level analyses during the study period before all children had reached the age of 24 months. Treatment with cortisol may influence levels of salivary cortisol,[16] but no family reported treatment with oral or inhaled corticosteroids. The results were adjusted for anthroposophic lifestyle. This lifestyle is influenced by ideas introduced by the Austrian philosopher Rudolf Steiner in the early 20th century and is characterized, for example, by giving birth at home, having an organic diet, restricting the use of antibiotics, antipyretics, and vaccinations as well as by several other features that may influence the allergy risk.[22] This adjustment in our models eliminates bias due to previously reported characteristics of children from these families concerning allergy and cortisol levels[18-21] and increases the generalizability of our results. Furthermore, there was no interaction effect between lifestyle group and salivary cortisol level on the outcomes. Variables in the models were chosen on the basis of on their considered importance for the development of allergic diseases in childhood and possible impact on the relationship between salivary cortisol level and allergies. Other factors, such as body weight of the child and the mother, have also been associated with childhood allergies, but because of the limited sample size, covariates in the models were restricted to those having the most substantial effect on asthma and allergy. For example, maternal allergy could potentially be considered a confounder, and we have therefore controlled for this factor (maternal sensitization) in our analyses as well as sensitization of the fathers.

In conclusion, our results show a clear association between raised levels of cortisol at age 6 months and the risk for sensitization and eczema during the first 2 years of life, which suggests that stress early in life may be of importance for the development of allergy in children.

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Clinical implications: In this prospective study, we demonstrate an association between increased levels of cortisol at age 6 months and the risk of sensitization as well as eczema during the first 2 years of life.

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