Risk factors associated with the development of atopic dermatitis among children in Yerevan: a case-control study.

Master of Public Health Thesis Project Utilizing Professional Publication Framework

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Abstract

Introduction: A number of studies shows that breastfeeding offers a safe and effective mode of protection against Atopic Dermatitis (AD) during the first years of life. However, the issue of protective effect of breastfeeding remains controversial. Therefore, further research is needed to confirm these associations.

Objectives: This study identifies the possible relationship between exclusive breastfeeding and early solid food diet and clinical manifestation of AD in children.

Design and methods: A case-control study was conducted among children aged from one to seven years in Yerevan. Cases were selected from Allergy Department of Republican Children's hospital in Yerevan. Controls were selected from pediatric district policlinics in Yerevan. Information regarding infant and family history and infant-feeding pattern was obtained from the mothers of children during telephone interviews. Eighty-five cases and 155 controls were interviewed. Descriptive analysis was used to determine characteristics of cases and controls. Multivariate logistic regression was used to control for potential confounding and effect modification.

Results: Exclusive breastfeeding less than 3 months increases the risk of AD over 2-fold (2.47, 95% CI 1.76-15.81). Initiation of solid food during the first 4 months increases the risk of AD over 3-fold (3.39, 95% CI 1.47-7.83).

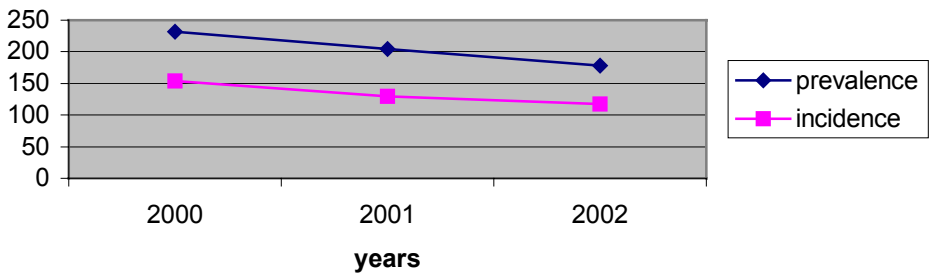
Conclusion: Early cessation of exclusive breastfeeding and early solid food diet are the risk factors for AD. Exclusive breastfeeding for at least 3 months and solid food avoidance during the first 4 months of life should be recommended for all infants to prevent development of AD. When exclusive breastfeeding is impossible solid food should be avoided during the first 4 months.

Introduction

I. Atopic Dermatitis

Atopic dermatitis (AD) is a chronic non-contagious disease that affects the skin. It is characterized primarily by intense itching and the development of papules, scaly lesions, fissures, and crusting. According to the results from a cross-sectional questionnaire survey conducted on random samples of schoolchildren aged 6 to 7 years and 13 to 14 years from centers in 56 countries throughout the world, the prevalence of AD for children aged 6 to 7 years ranged between 2% (Iran) and 16% (Japan, Sweden) and for those aged 13 to 14 years ranged between 1% (Albania) and 17% (Nigeria) (1). Higher prevalence of atopic dermatitis symptoms was reported in Australia and Northern Europe, and lower prevalence was reported in Eastern and Central Europe and Asia (1). Similar tendency was noted for symptoms of severe atopic dermatitis. In Armenia the prevalence of atopic dermatitis in children in 2000, 2001, and 2002 was 232.2, 203.5, and 178.3 per 100,000, and incidence was 154.2, 130.0, and 116.9 per 100,000 respectively (Figure 1).

Figure 1. Cases of Atopic Dermatitis in children aged from 0-14 years per 100,000 population in Armenia, 2000-2002.



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Atopic dermatitis impacts on quality of life. It can cause considerable disruption to the lives of children and their parents and involves significant financial cost for the families (2).

Children often experience multiple recurrences, repeated attempts at treatment, lowered self-esteem, impaired growth and development, loss of sleep, discipline problems, and multiple clinic and emergency department visits. In addition, the long-term diet that children must follow seems to be a stressor for children and their families (3).

Taking into account that the prevalence of atopic diseases is increasing throughout the world (1), prevention of allergic diseases should be a concern for all. To prevent and control allergic dermatitis, it is important to identify potential risk factors, as well as potential protective factors associated with this disease.

II. The etiology of Atopic Dermatitis

The etiology of AD is multifactorial. Researchers suspect that atopic dermatitis might be caused by environmental factors acting in people who are genetically predisposed to the disease. Heredity is an important biological risk factor in the development of immune sensitization and allergy (4, 5). A genetic study (6) suggest that atopies of parents might contribute to a twofold risk (2.16, 95% CI 1.58-2.96) for their children.

Researchers (7, 8, 9) suggest that early life events also play a role in the clinical manifestation of atopic diseases in children. Determinants for atopic diseases may be set already in early life when the infant's immune system hovers between sensitization and tolerance to allergens (7, 9). Small, but biologically significant, quantities of antigen are normally absorbed from the intestinal lumen (10). The mucousal barrier, which includes immune and other factors, limits antigen uptake (10). However, an infant has an immature gastro-intestinal tract (GIT) (10). The immature digestive system in early infancy contributes to increased permeability to foreign proteins in early life (10). Gastric acid production and enzyme secretion are reduced during the first 4 weeks of life (10). It is accompanied by immature or disordered intestinal peristaltic activity. The gut-associated lymphoid tissues are incompletely developed at birth. These factors may therefore contribute to the increased

permeability to foreign proteins in early life and can explain the enhanced antigen uptake in quantities sufficient to influence the immune system (10). Absorption of large molecules from the GIT may lead to immune system dysfunction and, as a result, to the development of AD (10, 11).

III. Protective effect of breast milk.

During this transient period of increased vulnerability, breast milk appears to possess factors that can assist in protecting infants (10, 11). Human colostrum provides passive protection to the intestinal surface (10, 11). Colostrum contains Secretory Immunoglobulin A (SigA) that can prevent allergen absorption by limiting contact between ingested antigen and the intestinal mucousal membrane (11, 12, 13). While epidermal growth factor in breast milk facilitates maturation of mucousal surface and limits exposure to antigen (10). Another possible protective mechanism for breastfeeding is its regulative effect on microbial flora in the GIT. Breastfed infants are more often colonized with lactobacilli and bifidobacteria compared to formula fed infants (14). Lactobacilli have been reported to reduce the risk for development of AD by 50% in children up to two years of age (15).

Several studies show that breastfeeding offers a safe and effective mode of protection against atopic dermatitis during the first years of life (8, 16, 17). Gdalevich and Mimouni (8) conducted a systematic review with meta-analysis of prospective studies and the summary odds ratio for the protective effect of breast-feeding was 0.68 (95% CI 0.52-0.88). Breastfeeding was more effective in children with a family history of atopy (OR = 0.58; CI, 0.41-0.92) than in combined populations (OR = 0.84; CI, 0.59-1.19) (8). However, the results of other studies show that this issue of protective effect of breastfeeding against the onset of atopic dermatitis during childhood remains controversial (18, 19, 20).

IV. Protective effect of delay of solid food initiation.

Fergusson and Horwood's (19) prospective cohort study suggested that breastfeeding practices had no effect on rates of atopic dermatitis: the apparent association between exclusive breastfeeding and reduced rates of atopic dermatitis reported in previous studies may be because exclusively breastfed infants were not exposed to early solid feeding rather than to any beneficial effect of breast milk itself. The purpose of digestion is to break foods into non-allergic simple sugars, amino acids and fatty acids. Infants are unable to do this efficiently until the age of six months or so (21, 22). The more mature a infant's digestive system is at the time of introduction of solid foods, the more likely it will be able tolerate them. Fergusson (20, 21) suggests that one of the best ways to prevent the development of allergies in children is to introduce solid foods according to a schedule which corresponds with the ability of the digestive system to fully digest and tolerate them.

Description of the study

I. Objectives/ Research questions/ Hypotheses

Further research is needed to confirm these associations for children in Yerevan. This study identified the possible relationship between exclusive breastfeeding and early solid food diet and clinical manifestation of atopic dermatitis in children.

Research questions addressed by this study are:

1. Is there an association between breastfeeding and decreased risk of development of atopic dermatitis among children in Yerevan?
2. Is early initiation of solid food associated with increased risk of development of atopic dermatitis among children in Yerevan?

Study variables

The dependent variable is the presence or absence of atopic dermatitis.

Independent variables of the study are duration of exclusive breastfeeding of infants and age of solid food initiation among infants.

Taking into account the findings of the related studies (6, 7, 9, 16) the following factors were considered intervening variables: gender of child, birth weight, gestational age, occurrence of atopic dermatitis, allergic rhinitis and asthma in the relatives of subjects, antibiotic use in the first year of life, and child's residence.

The primary hypothesis speculates that breastfeeding due to its immunoprotective potential is associated with decreased risk of symptoms of atopic dermatitis. The secondary hypothesis is that early solid food diet would affect the subsequent incidence of atopic dermatitis in children.

II. Study design:

A case-control design was used to identify potential risk factors associated with development of atopic dermatitis among children in Yerevan. The case-control design is especially useful when there is a need to study several risk factors, and when a study must be done relatively quickly and inexpensively. It is the most efficient design for rare diseases, and usually requires a smaller study population than a cohort study.

III. Sample size

Sample size was calculated using the formula from the textbook of Sempos and Kahan (23) assuming the number of controls two times higher than number of cases. Specifying the values for α=0.05, 1-β=0.8, prevalence in controls po=0.6 (full breastfeeding rate at age of 4 month in 2000 (24)), and delta (decrease in prevalence) =0.2 (11), the sample size for each group was the following: 80 cases and 160 controls (two sided). This sample size did not include allowance for non-respondents.

Definition of cases

In this study, cases are children aged from one to seven years old living in Yerevan who have been diagnosed with atopic dermatitis at the Allergy Department of Republican Children's Hospital in Yerevan, Armenia from January, 2001 to September, 2003.

Exclusion criteria: 1) patients with unverified diagnosis, 2) children older than 7 years at the time of data collection, 3) children not living in Yerevan.

Definition of controls

Controls are children aged from one to seven years living in Yerevan never diagnosed with atopic dermatitis.

IV. Case definition

To define cases, this study used the U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis, which are validated for use in an epidemiological setting (25). To be ascertained as a case of atopic dermatitis, a child should have an itchy skin condition and three or more of the following: history of flexural involvement, a history of dry skin, onset of rash under the age of 2 years, visible flexural dermatitis, and family or personal history of asthma/hay fever or pruritic skin condition.

V. Procedures involving the subjects of the research

The investigator reviewed medical register of Allergy Department for 2001-2003 period and recorded all eligible cases (110 cases) taking into account allowance for losses and non-respondents. Then contact information was obtained from the medical records including name, telephone number, and address. All cases were divided into groups according to their neighborhood.

Controls were selected from the district policlinics of Yerevan using multistage cluster sampling strategy with frequency matching of study groups by age, gender and residence. The investigator examined the medical records of prospective cohort to screen on eligibility.

VI. Interview instrument

Information regarding the infant and family history and infant feeding pattern was obtained using a structured written questionnaire, completed by mothers of the children. For

the development of the instrument of this study, the International Study of Asthma and Allergies in Childhood (26) instrument was used. The investigator supplemented it with additional questions related to the topic of interest. After the data collection protocol and research instrument were developed, they were tested to remove ambiguities and inconsistencies and revisions made. The first part of questionnaire contained demographic data, the second part information about birth weight, gestational age, infant nutrition, family atopy, and antibiotic exposure during the first year of life. Skin conditions questions were put at the end of questionnaire.

VII. Methods of intervention

The student investigator conducted telephone interviews with mothers of children, participating in the study. It took from 5 to 7 minutes to complete each interview. To minimize interviewer bias in assessment of exposure, the interviewer was not aware of the respondent's outcome status during interviews. Final ascertainment of cases and controls was done through questions about skin condition during the interview. During this study, 240 participants were interviewed (85 cases and 155 controls). Response rate was different for cases and controls, 75% and 95% respectively.

VIII. Ethical considerations

This study was revised and approved by a departmental Institutional Review Board (IRB) committee within the College of Health Sciences of American University of Armenia. Oral consent was received from the participants before starting the interview. The interviewees were fully informed about the study and its purpose, expected risks or benefits from participation, and confidentiality of information. All the information was treated in confidential manner. The names, telephone numbers, and addresses of participants were kept in special form separated from the questionnaires, and destroyed after data collection was completed.

IX. Data management and analysis

Data were entered on computer using SPSS software. STATA statistical program was used for analysis of data. Descriptive analysis was used to determine characteristics of cases and controls. Multivariate logistic regression models were used to control for potential confounding and effect modification. Independent variables such as exclusive breastfeeding of infants and solid food consumption were studied to find their associations with the development of atopic dermatitis among children. Factors without influence on the exposure or outcome were excluded from the multiple logistic regression model. All statistical tests were performed two-sided.

Results

The analysis was based on data from 155 controls and 85 cases. Both groups were comparable with regard to demographic characteristics such as age, gender, and place of residence due to group matching for those variables.

Table 1. The distribution on age, gender and residence among cases and controls.

	Cases		Controls	
Age categories (years)	N	%	N	%
1-2	34	40.00	63	40.65
3-4	20	23.53	40	25.81
5 and more	31	36.47	52	33.55
Gender				
Male	43	50.59	83	53.55
Female	42	49.41	72	46.45
Residence (districts)				
1	13	15.29	23	14.84
2	12	14.12	21	13.55
3	22	25.88	41	26.45
4	15	17.65	25	16.13
5	23	27.06	45	29.03
Total	85	100.00	155	100.00

The cases differed significantly from the controls with regard to the following variables: higher proportion of children with birth weight ≥4 kg, higher proportion of formula-fed infants, lower proportion of children breastfed exclusively for more than 4

months, lower proportion of children with delayed introduction of solid food after the forth months of life, higher proportion of antibiotics exposure during the first year of life, and higher atopic risk level (family history of atopy) among cases compared to controls (Table 2). The distribution of the following variables such as gestational age and breastfeeding duration was similar in both study groups.

Simple logistic regression was used to examine relationships between atopic dermatitis (AD) and study variables (Table 3). Statistically significant associations were found between AD manifestation in children and exclusive BF duration, age of solid food initiation, family history of allergy, antibiotics exposure during the first year of life, and birth weight of children when analyzed individually. There was 70 % decrease in risk of AD in breastfed children (OR=0.32; CI 0.1-1.01) although the result was of approached significance (p-value=0.053). The protective effect of breastfeeding increased with increasing breastfeeding duration, although the result was not statistically significant (Table 3).

Two study variables, exclusive breastfeeding duration and age at introduction of solid food, are not independent. Extended exclusive breastfeeding is associated with delayed solid food introduction. The investigator first performed separate analysis for each of the mentioned variables investigating the relationship with the development of AD in children. Then a new variable was created to examine combinative influence of exclusive breastfeeding duration and solid food initiation (Table 3).

Separate analysis of the relationship between exclusive breastfeeding duration and the development of AD in children during the first seven years of life revealed that the crude OR for exclusive breastfeeding up to the first 4 months of life or less was 2.04 (95% CI 1.16-3.60) compared to exclusive breastfeeding for more than 4 months (Table 3). The crude OR for exclusive breastfeeding up to the second month of life or less was 3.16 (95% CI 1.60-6.23) and for exclusive breastfeeding from 3 to 4 months was 1.44 (95% CI 0.75-2.75)

compared to exclusive breastfeeding for more than 4 months. It seems that the longer the infant is breastfed exclusively, the lower risk for the development of AD. Unconditional multivariate logistic regression was used to control for potential confounders and examine for effect modification factors. Family history of allergy, birth weight, and the surroundings of the child's house during the first year of life confounded the relationship between AD and exclusive breastfeeding duration. There was an interaction between allergy in mothers and allergy in fathers. After adjustment for atopic risk factors, the surroundings, and birth weight and taking into account the interaction between allergy in mothers and allergy in fathers the OR for exclusive breastfeeding for the first 2 months of life or less decreased to 2.47 (95% CI 1.07-5.72) and for exclusive breastfeeding from 3 to 4 months became 0.94 (0.41-2.15) compared to exclusive breastfeeding for more than 4 months (Table 4). Atopic risk level, surroundings without parks or gardens and antibiotics exposure were strongest risk factors for AD (Table 4). Allergy of father seemed to be stronger risk factor for the development of AD in children than allergy of mother. Highest risk (OR=14.94; 95% CI 2.80-79.70) for AD was for children from families with double atopic risk (both parents have allergy).

The separate analysis investigating the relationship between the development of AD and solid food initiation revealed that the crude OR for solid food initiation during the first 4 months of life was 3.94 (95% CI 2.02-7.67) compared to initiation after 4 months. Avoidance of solid food during 5 and 6 months of life did not show any evidence of additional protection from development of AD in children. There was a dose-response relationship between number of solid food item added to diet of children and manifestation of AD during the first seven years of life. Risk for AD increases 2 times with every additional solid food item initiated during the first 4 months of age. Atopic risk, birth weight of a child, surroundings of a child's home during the first year of life, and antibiotics exposure confounded the relationship between AD and solid food consumption. These factors as well as an interaction

between allergy in mothers and allergy in fathers were taken into consideration when choosing a final model. After controlling for confounding and taking into account the interaction term the OR of solid food initiation during the first 4 months of life compared to solid food initiation after 4 months decreased to 3.39 (95% CI 1.47-7.83) (Table 5).

The analysis examining the relationship between the development of AD and infant feeding pattern (combinative variable of exclusive breastfeeding duration and solid food initiation) showed that the crude OR for exclusive breastfeeding from 3 to 4 months and solid food initiation after 4 months was 1.03 (95% CI 0.64-2.66), for exclusive breastfeeding for 2 months or less and solid food initiation after 4 months was 1.44 (95% CI 0.64-3.24), for exclusive breastfeeding from 3 to 4 months and solid food initiation before 4 months the crude OR was 1.85 (95% CI 0.71-4.81), and for exclusive breastfeeding for 2 months or less and solid food initiation before 4 months was 14.37 (95% CI 4.46-46.30) compared to exclusive breastfeeding for more than 4 months and solid food initiation after 4 months (Table 3).

Family history, birth weight, surroundings, and antibiotics exposure confounded the relationship between the development of AD and infant feeding pattern. After adjustment for family history, birth weight, surroundings, and antibiotics exposure and taking into consideration the interaction between allergy in mothers and allergy in fathers the OR for exclusive breastfeeding from 3 to 4 months and solid food initiation after 4 months became 0.77 (95% CI 0.30-1.98), for exclusive breastfeeding for 2 months or less and solid food initiation after 4 months was 1.15 (95% CI 0.41-3.19), for exclusive breastfeeding from 3 to 4 months and solid food initiation before 4 month the crude OR was 1.23 (95% CI 0.36-4.14), and for exclusive breastfeeding for 2 months or less and solid food initiation before 4 months decreased to 10.65 (95% CI 2.70-41.89) compared to exclusive breastfeeding for more than 4 months and solid food initiation after 4 months (Table 6).

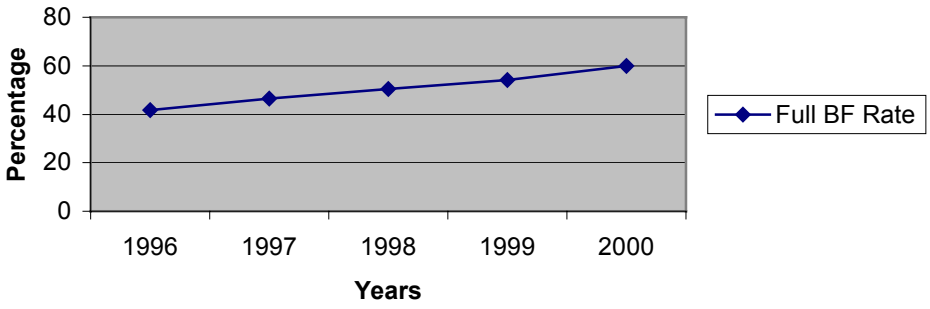
Discussion

This study indicates that exclusive breastfeeding is a protective factor against development of AD during the first seven years of life. The risk for AD is 2.5 times higher for children breastfed exclusively for 2 months or less compared to children breastfed for more than 4 months. There is a strong association between exclusive breastfeeding and the development of AD. Analysis of the relationship between both breastfeeding duration and exclusive breastfeeding duration and the development of atopic dermatitis revealed that the longer breastfeeding duration the lower the risk for AD (dose-response). The result is consistent with observations in previous studies and shows the protective effect of exclusive breastfeeding against development of AD among children (8, 16, 17). The protective effect of exclusive breastfeeding particularly increases in children with a family history of atopy. This study could not confirm the results of the New Zealand study (21), which showed that exclusive breastfeeding itself has no protective effect against the development of AD among children. Also this study did not confirm the results of Bergman's study (18) that speculated that breastfeeding duration increases the risk for the development of AD in children. However, a difference in study designs, data collection methods, and controlling for different factors makes comparison of the results difficult.

The results of this study are consistent with existing biological knowledge. The protective effect of exclusive breastfeeding may operate through several mechanisms such as regulation of microbial flora, lower allergen absorption, and provision of immunomodulatory and anti-inflammatory components in human milk (11). The observed association between exclusive breastfeeding duration and the development of AD is not due to confounding because the investigator adjusted for several possible confounders such as family history, birth weight, surroundings of the child's house during the first year of life, and antibiotics

exposure and controlled for effect-modification during analysis. Also group matching on age, gender and residence allowed eliminating of the confounding effect of these factors on association between AD development and exclusive breastfeeding as well as solid food initiation. However, it can not be ruled out that other confounders can be responsible for observed association. The results of the study are consistent with statistical data. Figure 1 and Figure 2 show data regarding atopic dermatitis rates in children and full breastfeeding rates in children in Armenia respectively. The rate of full breastfeeding is increasing while AD rate is decreasing.

Figure 2. Full BF Rate at the age of 4 months in Armenian children, 1996-2000



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A strong association observed between exclusive breastfeeding duration and development of AD as well as consistency with observations in previous studies, with biological knowledge, and with statistical data showed that association between exclusive breastfeeding and AD is due to a causal relationship.

There is a strong association between early solid feeding during the first 4 months and risk of AD: children exposed to solid food diet during the first 4 months had risk of AD which was 3.5 times higher those of children who were not introduced to solid food by age four months. The risk for AD increased in 10 times for children breastfed exclusively for 2

months or less and with early solid food diet during the first 4 months of life compared to children breastfed exclusively for more than 4 months. The investigator observed positive interaction of these two risk factors because OR of AD in the presence of those factors differed from OR expected to result from their individual effect. There was a dose-response relationship between number of food items in diet of children during the first 4 months of life and AD manifestation. The risk of AD increased with every additional item in the infant's diet. The results are consistent with the related studies (20, 21, 22) that speculated that early solid food initiation could be a risk factor for the manifestation of AD in children. Fergusson (22) speculates that solid food initiation should be delayed until 6 months of age. Current study did not find any additional protective effect of avoidance of solid food during the fifth and sixth months of age. However, differences in study design may contribute to the different results.

The findings of this study are coherent with biological knowledge: the more mature the infant's digestive system at the time of solid food introduction the more likely they will tolerate them (10). The investigator has taken into consideration other possible explanations for observed association between solid food initiation and the development of AD and adjusted for them. All these factors show that the relationship between solid food initiation and the development of AD are causal.

A family history of atopy proved to be strongest risk factor for AD. The risk of AD increases from single level of atopic risk when only one of the parents has atopy to double level when both parents have atopic diseases. Another strong risk factor is antibiotic exposure during the first year of life. During this study investigator relied on the mother's report of antibiotics exposure. It is assumed that term "antibiotics" is well understood by mothers of children in Yerevan. Investigator verified the answer of mothers about antibiotic exposure during interview by asking them to name the drugs given to the child during the first year of

life. The risk is higher in children with two or more courses of antibiotics compared to those exposed to only one course. The effect of antibiotics was time-dependent, showing higher risk of AD in children exposed to antibiotics during period from one to six months of age compared to children exposed after six months of age. The data are consistent with the results of previous study (9) investigating the relationship between AD development in children and early antibiotic exposure and supports theoretical knowledge. The composition of the gut microflora early in life is an important determinant of atopic status. Exposure to antibiotics might be a risk factor for the development of allergy because it causes major disruptions to the gut microflora.

Limitations of the study

1. One of the limitations can be non-coverage or group underrepresentation in the study. Telephone interview design does not give everyone a chance of being including in the study because the sample includes only people with telephones. However, telephone interview approach was chosen as the most feasible assuming the wide coverage of population in Yerevan with telephones.
2. There was different response rate for cases and controls, 75% and 95 % respectively. The reasons for non-response were subjects moving from the previous residence, change of the telephone numbers, no answers, and busy line. The interviewer tried to keep non-response to a minimum making repeated calls during the study. There were only two refusals to participate.
3. Misclassification of cases and controls can be limitation of the study. However, the final ascertainment of cases and controls was done through skin condition questions validated for use in epidemiological settings.
4. Another limitation can be misclassification of the exposure. This may result from recall bias. The mothers of children with AD might remember more detailed information about exposure, while the mothers of healthy children trend to underestimate exposure.

5. Other possible confounders not adjusted for during current study may influence observed associations.

Conclusion

Early cessation of exclusive breastfeeding and early solid food diet are the risk factors for AD. Exclusive breastfeeding for at least 3 months and solid food avoidance during the first 4 months of life should be recommended for all infants to prevent development of AD. Particularly it concerns children with family history of atopy. In those cases when exclusive breastfeeding beyond 3 months is impossible or in cases of artificially fed infants, solid food should be avoided during the first 4 months of life, particularly among those who are at hereditary risk.

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Table 2. Distribution on surroundings of child's home, birth weight, gestational age, infant feeding, family history of allergy, and antibiotics exposure during first year of life among cases and controls.

Description of variables	Cases		Controls		χ^2 (pr)
The surroundings during the first year of life	N	%	N	%	
With many parks or gardens	21	24.7	20	12.9	19.6 (0.00)
With few parks or gardens	53	62.3	132	85.2	
With no parks or gardens	11	12.9	3	1.9	
Birth weight (gr.)					
2,500-3,999	73	85.9	139	89.7	7.1 (0.029)
< 2,500	2	2.3	10	6.45	
≥4,000	10	11.8	6	3.9	
Gestational age					
Term	80	94.1	150	96.8	2.2 (0.33)
Preterm	4	4.7	5	3.2	
Postterm	1	1.2	0	0.00	
Infant feeding					
Breastfeeding (BF)	77	90.6	150	96.8	4.1 (0.043)
Artificial feeding	8	9.4	5	3.2	
Breastfeeding duration (months)					
≤4	25	32.5	38	25.3	1.99 (0.575)
5-6	15	19.5	27	18.0	
7-12	19	24.7	39	26.0	
>12	18	23.4	46	30.7	
Exclusive BF duration (months)					
≤2	25	32.5	25	16.7	8.5 (0.037)
3-4	28	36.4	56	37.3	
5-6	19	24.7	56	37.3	
>6	5	6.5	13	8.7	
Solid food initiation (months)					
≤2	3	3.53	0	0.00	18.3 (0.000)
3-4	26	30.6	18	11.6	
5-6	30	35.3	74	47.7	
>6	26	30.6	63	40.6	
Allergy in parents					
No allergy	27	31.8	134	86.45	74.8 (0.000)
Allergy in mothers	28	32.9	12	7.7	
Allergy in fathers	22	25.9	7	4.5	
Allergy in both parents	8	9.4	2	1.3	
Antibiotics exposure during first year of life					
No exposure	45	52.9	118	76.1	13.5 (0.001)
One course	30	35.3	28	18.1	
Two courses or more	10	11.8	9	5.8	
Infant feeding pattern (combined)					
Exclusive BF duration >4, Solid food initiation >4	24	28.2	69	44.5	
Exclusive BF duration 3-4, Solid food initiation >4	19	22.3	42	27.1	
Exclusive BF duration ≤2, Solid food initiation >4	13	15.3	26	16.8	10.3 (0.006)
Exclusive BF duration 3-4, Solid food initiation ≤4	9	10.6	14	9.0	
Exclusive BF duration ≤2, Solid food initiation ≤4	20	23.5	4	2.58	

Table 3. The relationship between development of AD among children and each of covariates when analyzed individually in a simple logistic regression model.

Description of variable	Unadjusted OR (95%CI)
Birth weight (gr.)	
2,500-3,999 (reference)	
< 2,500	0.38 (0.08-1.8)
≥4,000	3.2 (1.1-9.1)*
Gestational age	
Term (reference)	
Preterm	1.5 (0.39-5.7)
Breastfeeding	
No (reference)	
Yes	0.32 (0.1-1.01)
BF duration (months)	
≤ 4 (reference)	
5-6	0.84 (0.38-1.89)
7-12	0.74 (0.35-1.56)
>12	0.59 (0.28-1.25)
Exclusive BF duration (months)	
>4 (reference)	
3-4	1.44 (0.75-2.75)*
≤2	3.16 (1.60-6.23)
Exclusive BF duration (months)	
>4 (reference)	
≤4	2.04 (1.16-3.60)
Solid food consumption (months)	
>4 (reference)	
≤4	3.94 (2.02-7.67)**
Allergy in parents	
No allergy (reference)	
Allergy in mother	11.58 (5.24-25.58)**
Allergy in father	15.59 (6.06-40.16)**
Allergy in both parents	19.85 (3.99-98.69)**
Antibiotics exposure during the first year	
No (reference)	
Yes	2.83 (1.61-5.00)**
Infant feeding pattern (combined)	
Exclusive BF duration >4, Solid food initiation >4 ref	
Exclusive BF duration 3-4, Solid food initiation >4	1.30 (0.64-2.66)
Exclusive BF duration ≤2, Solid food initiation >4	1.44 (0.64-3.24)
Exclusive BF duration 3-4, Solid food initiation ≤4	1.85 (.71-4.81)
Exclusive BF duration ≤2, Solid food initiation ≤4	14.37 (4.46-46.30)**

Note: AD= atopic dermatitis; BF= breastfeeding; OR= odds ratio; CI= confidence interval.
Significance level: *P<.05; **P<.005.

Table 4. Logistic regression models of AD by exclusive BF duration, allergy in parents, birth weight, and surroundings of child's home during the first year of life among children in Yerevan.

	Model1	Model2
Description of variable	OR (95%CI)	OR (95%CI)
Exclusive BF duration (months)		
>4 (reference)		
3-4	1.04 (0.47-2.30)	0.94 (0.41-2.15)
≤2	2.55 (1.12-5.78)*	2.47 (1.07-5.72)*
Allergy in mothers		
No (reference)		
Yes	11.30 (4.94-25.87)**	9.80 (4.19-22.86)**
Allergy in fathers		
No (reference)		
Yes	15.22 (5.63-41.13)**	14.59 (5.29-40.20)**
Interaction : Allergy in mothers and Allergy in fathers	0.10 (0.01-0.74)*	0.10 (0.01-0.82)*
Birth weight (gr.)		
2,500-3,999 (reference)		
< 2,500	0.19 (0.30-1.19)	0.22 (0.03-1.41)
≥4,000	2.33 (0.65-8.29)	2.37 (0.65-8.62)
The surroundings during the first year of life		
With few parks or gardens (reference)		
With many parks or gardens		1.95 (0.82-4.62)
With no parks or gardens		6.20 (1.35-28.34)*

Note: AD= atopic dermatitis; BF= breastfeeding; OR= odds ratio; CI= confidence interval.
Level of significance: *P<.05; **P<.005.

Table 5. Multivariate logistic regression models of AD by solid food initiation pattern, allergy in parents, birth weight, surroundings of the child's home, and antibiotics exposure.

	Model1	Model2
Description of variable	OR (95%CI)	OR (95%CI)
Solid food consumption (months)		
>4 (reference)		
<=4	3.39 (1.47-7.83)*	3.69 (1.62-8.4)*
Allergy in parents		
No allergy (reference)		
Allergy in mother	9.96 (4.2-25.0)**	10.20 (4.32-24.05)**
Allergy in father	12.89 (4.5-38.0)**	12.29 (4.48-33.63)**
Allergy in both parents	16.39 (2.97-90.52)*	19.04 (3.60-100.62)*
Birth weight (grams)		
2,500-3,999 (reference)		
<2,500	0.33 (0.04-2.69)	0.33 (0.05-2.22)
≥4,000	3.10 (0.97-5.57)	2.61 (0.67-10.07)
The surroundings during the first year of life		
With few parks or gardens (reference)		
With many parks or gardens	2.32 (0.97-5.57)	2.17 (0.92-5.12)
With no parks or gardens	6.33 (1.20-33.28)*	5.02 (1.05-24.04)*
Antibiotics exposure during the first year		
No (reference)		
Yes		3.01 (1.46-6.23)**

Note: AD= atopic dermatitis; BF= breastfeeding; OR= odds ratio; CI= confidence interval.
Significance level: *P<.05; **P<.005.

Table 6. Multivariate logistic regression model of AD by infant feeding pattern, allergy in parents, surroundings of the child's home, birth weight, and antibiotics exposure.

	Model1
Description of variable	OR (95%CI)
Infant feeding pattern (months)	
Exclusive BF duration >4, Solid food initiation >4 ref	
Exclusive BF duration 3-4, Solid food initiation >4	0.77 (0.30-1.98)
Exclusive BF duration ≤2, Solid food initiation >4	1.15 (0.41-3.19)
Exclusive BF duration 3-4, Solid food initiation ≤4	1.23 (0.36-4.14)
Exclusive BF duration ≤2, Solid food initiation ≤4	10.65 (2.70-41.89)**
Allergy in parents	
No allergy (reference)	
Allergy in mother	9.51 (3.84-23.60)**
Allergy in father	13.92 (4.80-40.44)**
Allergy in both parents	14.69 (2.63-82.12)**
The surroundings during the first year of life	
With few parks or gardens (reference)	
With many parks or gardens	2.53 (1.02-6.29)*
With no parks or gardens	5.67 (1.04-31.13)*
Birth weight (grams)	
2,500-3,999 (reference)	
<2,500	0.27 (0.03-2.60)
≥4,000	3.48 (0.82-14.79)
Antibiotics exposure during the first year	
No (reference)	
Yes	3.09 (1.47-6.47)**

Note: AD= atopic dermatitis; BF= breastfeeding; OR= odds ratio; CI= confidence interval.
Significance level: *P<.05; **P<.005.

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Appendix A

American University of Armenia
College of Health Sciences
Master of Public Health Program

Investigation of the potential risk factors associated with the development of atopic dermatitis.

Questionnaire

ID_ _ _ _ _ _

Demographic characteristics

Dm1. What is the gender of your child? 1. Boy
2. Girl

Dm2. When was your child born? _____ (Day/Month/Year)

Dm3. Was your child born in Yerevan? 1. Yes

2. No

a. If no, where was your child born? _____

Dm4. What is the name of your child street of residence? _____

Dm5. How would you describe the surroundings of your child's home?

	1. At present	2. During the child's first year of life
1. open spaces or fields nearby	՞	՞
2. with many parks or gardens	՞	՞
3. with few parks or gardens	՞	՞
4. with no parks or gardens	՞	՞

Dm6. For how long did the child's mother attend school or professional training?

	Mother
1. School	_____years
2. College/University	_____years

Dm7. Who has answered this questionnaire? 1. Mother

2. Other person, specify _____

Dm8. When was the questionnaire answered? _____
(Day/Month/Year)

Early days

ED1. How much did your child weight at birth?
1. < 2500 g 2. 2500-2999 g 3. 3000-3999 g 4. >=4000 g 66. Do not remember

ED2. Was your child born on time or not? Was your child born within 3 weeks of the calculated date?

1. Yes 2. No, > 3 weeks early 3. No, > 3 weeks late 66. Do not remember

ED3. Is your child a twin? 1. Yes
2. No

Infant feeding pattern

IF1. Was your child ever breastfed? 1. Yes ⇒ IF3
2. No⇒ IF2
IF2. If no, what kind of food was initiated? (check all that apply) ⇒ IF5
a) infant formula
b) animal milk
c) yogurt
d) Narine
66. Other, specify_____
IF3. If yes, for how long?
1. ≤ 4 months 2. 5-6 months 3. 7-12 months 4. > 1 year 66. Do not remember

IF4. If yes, how long was your child breastfed without adding other foods or juices?⇒IF5
1. ≤ 2 months 2. 3-4 months 3. 5-6 months 4. > 6 months 66. Do not remember

IF5. When solid food was initiated?
1. ≤ 2 months 2. 3-4 months 3. 5-6 months 4. > 6 months 66. Do not remember

IF6. How old was your child when you first gave him food other that breast milk? (response category "never" (4) means "did not give during the first year of life")
a. Infant formula
1. ≤ 4 months 2. 5-6 months 3. > 6 months 4. Never 66. Do not remember
b. Animal milk
1. ≤ 4 months 2. 5-6 months 3. > 6 months 4. Never 66. Do not remember
c. Yogurt
1. ≤ 4 months 2. 5-6 months 3. > 6 months 4. Never 66. Do not remember
d. Narine
1. ≤ 4 months 2. 5-6 months 3. > 6 months 4. Never 66. Do not remember
e. Porridge
1. ≤ 4 months 2. 5-6 months 3. > 6 months 4. Never 66. Do not remember

f. Vegetables

1. ≤ 4 months	2. 5-6 months	3. > 6 months	4. Never	66. Do not remember

g. Fruits

1. ≤ 4 months	2. 5-6 months	3. > 6 months	4. Never	66. Do not remember

h. Meat

1. ≤ 4 months	2. 5-6 months	3. > 6 months	4. Never	66. Do not remember

i. Eggs

1. ≤ 4 months	2. 5-6 months	3. > 6 months	4. Never	66. Do not remember

j. Cheese or cottage cheese

1. ≤ 4 months	2. 5-6 months	3. > 6 months	4. Never	66. Do not remember

k. Bread or biscuit

1. ≤ 4 months	2. 5-6 months	3. > 6 months	4. Never	66. Do not remember

Diseases

D1. Has the child's mother ever had any of the following diseases?

(tick as many boxes as apply)

a) Asthma
b) Hay fever
c) Food allergy
d) Atopic dermatitis (eczema)
e) Other allergic conditions

Specify_____

D2. Has the child's father ever had any of the following diseases?

(tick as many boxes as apply)

a) Asthma
b) Hay fever
c) Food allergy
d) Atopic dermatitis (eczema)
e) Other allergic conditions

Specify_____

Ant1. In the first 12 months of life, did your child have any antibiotics?

1. Yes⇒Ant2
2. No

Ant2. If yes, during what time period? a) During the first month

(tick as many boxes as apply) b) 1-6 months

c) 6-12 months

Ant3. If yes, how many courses in the first 12 months?

1. one course 2. two courses 3. three courses 4. four and more courses 66. Do not remember

Questionnaire on skin symptoms

Sk1. Have your child ever had an itchy rash which was coming and going for at least six months? 1. Yes 2. No⇒Sk7

Sk2. Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes? 1. Yes 2. No

Sk3. At what age did this rash first occur?

1. < 2 years 2. Age 2-4 years 3. Age 5 or more 66. Do not remember

Sk4. Has your child had a history of a generalized dry skin? 1. Yes 2. No

Sk5. Has your child had this itchy rash at any time in the last 12 months? 1. Yes 2. No ⇒Sk7

Sk6. Has this rash cleared completely at any time during the last 12 months? 1. Yes 2. No

Sk7. In the last 12 months, how often, on average, has your child been kept awake at night by this itchy rash?

1. Never in the last 12 months

2. Less than one night per week

3. One or more night per week

Sk8. Has the child ever had any of the following diseases?

(tick as many boxes as apply)

a) Asthma

b) Hay fever

c) Food allergy

d) Atopic dermatitis (eczema)

f) Other allergic conditions
Specify_____

Sk81. If yes, was this diagnosed by a doctor?

1. Yes
2. No

Thank you

Appendix B

American University of Armenia

Department of Public Health

Institutional Review Board/Committee on Human Research

CONSENT FORM TEMPLATE

Title of Research Project: Investigation of the potential risk factors associated with the development of atopic dermatitis in Armenian children.

CHR#

The student of the Public Health Department of the American University of Armenia is conducting a study to understand more about the increasing problem of skin symptoms in children. The investigator intends to look at why some children develop atopic dermatitis others do not. Children with atopic dermatitis and children without known atopic dermatitis are being surveyed. For this purpose parents/caregivers are invited to take part in this study. For each child, a parent/caregiver is being asked to complete a written questionnaire, which includes questions about family history, infant care and infant/child feeding. It will take approximately 6 minutes. A parent also is being asked having child's medical records reviewed.

Explanation of Research Project:

Risks/Discomforts: There is no special risk involved in being a participant. Investigator has tried not to include sensitive questions in the questionnaire.

Benefits: Even though you and your child will not benefit individually from the participation in this study it is expected that other children will benefit from the knowledge gained from the study.

Confidentiality:

The information collected about your child will be treated in a confidential manner and your child will not be personally identified in the reporting of the results. Up to 240 parents/caregivers will be interviewed and your answers will be combined with theirs to make totals. Although this study will collect specific identifiers such as names, telephone numbers, and addresses to manage sample design and collecting data, this information will be kept in a special form strictly separated from the questionnaires. There will be no identifiers on the questionnaires. Once data collection will be done the form with names, telephone numbers and addresses will be destroyed. Your individual responses will only be accessible by the study investigators. After the data will be transferred to the computer files original papers will be kept in a secure area and stored for 3-years. After that time, they will be destroyed. Summary information and grouped responses that do not permit the identification of individuals will be submitted to the Public Health Department of the American University of Armenia and, possibly, can be published in professional journals.

Voluntariness: Your participation is entirely voluntary and you may refuse to answer any question if you choose, or may withdraw your consent to participate at any time without penalty or without any way affecting the health care your child receives.

Whom to Contact: You may ask any question you have about this study at this time. If you have further questions about this study you may call the person in charge of the study, **Yelena Amirkhanyan at phone number: (3741) 512568 and Michael Thompson** at **phone number: (3741) 512592.**

Appendix C

List of appropriate journals where this study might be published:

1. “Clinical and Experimental Allergy”
2. “Pediatric Allergy and Immunology”
3. “Journal of Allergy and Clinical Immunology”