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MEDICAL UNIVERSITY "PROF. DR. P. STOYANOV"- VARNA
FACULTY OF PUBLIC HEALTH
DEPARTMENT OF HYGIENE AND EPIDEMIOLOGY

Miglena Iordanova Marinova - Achkar, MD

The importance of breastfeeding for the health of children up to 3 years of age, born in families at high risk for allergy

Abstract of a Dissertation

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Scientific Supervisors: Prof. Rouzha Pancheva, MD, PhD
Assoc. Prof. Natalya Usheva, MD, PhD

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The dissertation contains 154 pages and is illustrated with 6 figures, 31 tables and 11 appendices. Cited are 306 literary sources from which 31 in Cyrillic and 275 in Latin.

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External members:

- Prof. Maya Krusteva - Vilmosh, MD, PhD
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C O N T E N T

ABBREVIATIONS USED

AD – atopic dermatitis

ADHD – attention deficit hyperactivity disorder

AR - allergic rhinitis

BMI - body mass index

CMPA – cow milk's protein allergy

FA – food allergy

HA – height-for-age

IF – infant formula

IQ - intelligence quotient

IBMA – index of body mass for age

NC - nasoconjunctivitis

ND - neurodevelopment

CoMiSS – Cow milk's related symptom score (a tool for clinical evaluation of symptoms of cow's milk protein allergy)

DP -3 - Development profile -3

SCORAD – SCORing of atopic dermatitis (visual scoring system for assessing the severity of atopic dermatitis)

WA – weight-for-age

WH – weight-for-height

WHO – World Health Organization

I. INTRODUCTION

Nowadays, allergic diseases are among the most common health condition, affecting up to 30% of children. The main theories about the origin of allergic diseases relate to changes in nutrition along with general environmental changes that shape the type of host immune response during a critical period of life to an allergic-type immune response. The trend observed in recent years towards an increase in the frequency of allergic diseases is associated with environmental pollution from chemical substances, presence of food allergens, uncontrolled use of medicines, and changes in the lifestyle of the population. Preventive strategies are especially important for children at high risk of developing allergies - when one or both parents are allergic. It is suggested that the best opportunities for prevention are in the time frame between conception and the first six months of life.

Globally, mother's breast milk is recognized as the "gold standard" in infant nutrition, and if it is in sufficient quantity, it completely satisfies the child's needs for all the nutrients necessary for normal growth and development up to 6 months of age. Breastmilk reduces the risk of infections by improving immune system functions and is thought to reduce the risk of food allergy (FA), especially to cow's milk protein. Breast milk should be the main source of nutrition during the critical period of metabolic and immune programming and because of its effects on intestinal function. The large number of bioactive factors contained in it influence the intestinal immune maturation of the child. Breastfeeding affects the gut microbiota and subsequent immune development, as human milk contains not only nutrients, but also polysaccharides, cytokines, proteins and other components that can epigenetically modulate the child's innate and adaptive immune responses during the early stages of life. Breastfeeding has short-term (reduced morbidity and mortality from neonatal infections) and long-term benefits (reduced risk of socially significant diseases - hypertension, type 2 diabetes), increased IQ (intelligence quotient) and better educational achievements later in life.

Children with a family history of allergies have a higher health risk not only for other allergic diseases, such as allergic asthma and allergic rhinitis, but also for somatic and psychosomatic diseases like attention deficit hyperactivity disorder (ADHD) etc. Many of the results of researches on the relationship between nutrition and the health of the young child are controversial, because various confounding factors during breastfeeding can influence its health effects on the growing child's organism, especially with a family predisposition - maternal diet during lactation, the age of introducing solid foods, early contact with animals, socio-economic and demographic status of the family, duration of breastfeeding. All of them complexly modulate children's health. It remains an open question to what extent breast milk, if other factors being equal, is decisive for conducting these processes, especially during the first years of life.

II. PURPOSE

To study the influence of breastfeeding on the growth, development and health of children of early childhood (including physical development and neurodevelopment (ND), acute morbidity, manifestation of allergies) from families with evidence of atopy.

III. TASKS

1. To evaluate the influence of breastfeeding on the growth of children born with family history of allergy.

2**.** To measure the relationship between breastfeeding and acute morbidity of these children;

3. To track the manifestations of allergy and their relationship with milk feeding in the studied group of children;

4. To evaluate the ND of children with history of allergy, as well as the influence of breastfeeding on it;

IV. FORMULATED WORKING HYPOTHESES:

- Breastfeeding affects favorably the indicators of physical and neurodevelopment of children with family history of allergy during the first years of life;
- The frequency of acute diseases and the occurrence of atopic conditions is lower in breastfed children during early childhood.

V. MATERIAL AND METHODS

1. Material

Object of the scientific research: The sample was formed in the Multidisciplinary Hospital for Active Treatment "St. Anna" - Varna through an invitation sent to 1210 women in labor, of which 156 accepted to participate in the monitoring. Potential participants completed a questionnaire assessing medically confirmed allergy in the child's mother, father, or sibling. Due to non-compliance with the terms of the study or withdrawal of consent, 36 respondents dropped out of the study. 120 children from families at high risk for allergy (54 girls and 66 boys) completed the study protocol.

Design, timing and organization of scientific research

The prospective cohort study among children with a family history of allergy was conducted in the period 2017-2021. Participants were included in the study after obtaining written informed consent from their parents or caregivers. At the beginning of the study, the child was examined and a questionnaire was filled out by the parents/caregivers to determine whether the child met the conditions for participation, according to the following criteria:

Inclusion criteria*:*

- Healthy newborns with birth weight over 2500 g;
- Morphological maturity – over 37 gestational weeks;
- Age - from 0 to 4 days at the start of the study;

- Family history of allergic diseases of mother, father or siblings;
- Signed informed consent from parents /guardians;
- Willingness of parents to participate in the study and to monitor their child's health status

Excluding criteria:

- Accompanying diseases at the beginning of the study;
- Prematurity and immaturity at birth ;
- Birth trauma;
- Asphyxia at birth;
- Genetic diseases;
- Presence of malformations;
- Refusal of parents to participate in monitoring their child's health status

Conducting the fieldwork:

- Information about the purpose and methods of the study was given to the parents and they were asked questions regarding current and past history of doctor-diagnosed allergies and the type of allergies in the family - asthma, allergic nasoconjunctivitis (NC), urticaria, food allergy, atopic dermatitis (eczema).
- Parents were asked to fill out a questionnaire regarding the socio-demographic characteristics of the family, their education, ethnicity, mode of conception, course on pregnancy, the presence of harmful habits, living with a pet, smoking, etc.
- Anthropometric parameters of the child such as weight, height and head circumference were measured at birth and at 2, 4, 6 months of age and a repeated clinical examination was performed using visual schemes for the assessment of atopic dermatitis (SCORAD) and questionnaires for potential manifestations of allergies (COMISS). Children with pronounced atopic conditions were additionally consulted with a pediatrician and a diagnostic process was carried out to clarify the allergy data - prick-test, elimination-provocation according to a standard protocol;
- Information about breastfeeding practices, height and weight at 1 year of age and at the time of survey, about the frequency and duration of acute illnesses and the occurrence of doctor-diagnosed allergies was obtained between 2 and 3 years of age through a direct individual survey until the end of follow-up;
- A validated questionnaire was used to assess the ND of the children in the follow-up group and compare it with the standards for the respective age.

2. Methods

2.1. Documentary method - the present work is based on an analysis of Bulgarian and foreign literature, including books, textbooks, articles, publications related to the subject of dissertation. In the course of the study, a targeted, topic-oriented analysis of more than 200 publications available in PubMed, SCOPUS and Science Direct was carried out. Some normative documents and methodological guidelines concerning the feeding of children in infancy and early childhood were used too.

2.2. Survey method

Direct individual questionnaire surveys were conducted, and questionnaire cards were developed and used, including the following signs of observation:

- Demographic and socio-economic indicators of the parents – gender, age, education, harmful habits (smoking), place of residence (city/village), ethnicity, number of family members, data about a pet in the house, types of allergies in the family ;
- Mode of conception, course of pregnancy and way of delivery ;
- Demographic and anthropometric indicators of the child - gender, age, height and weight at birth, at 1 year of age, at 2-3 years of age ;
- Completion of a visual scale for the assessment of data on atopic dermatitis (SCORAD) and a questionnaire for the assessment of potential occurrence of allergies (CoMiSS);
- Feeding practices of the child - periods of exclusive breastfeeding, total duration of breastfeeding, mixed and substitute feeding;
- Health status of the child - frequency and duration of acute diseases of the respiratory, digestive, urogenital systems, sensory organs, etc.; manifestations of allergies;
- Assessment of the child's ND - by completing a validated questionnaire Development Profile-3 (DP-3).

DP-3 methodology (rating scale for assessing child development in five key functional areas) uses information provided by parents/caregivers to provide a standard assessment of development in the following areas:

- Physical development;
- Adaptive behavior;
- Socio-emotional development;
- Cognitive development;
- Communication

The results of the five functional scales form the general developmental index, which is mapped to different ranges of interpretation. The DP-3 enables a rapid assessment of children's typical development and identification of delays in one or more of the assessed areas. The instrument is designed to assess the development of children from birth to 12 years and 11 months.

2.3. Clinical methods - anamnesis, status, anthropometric measurements at birth, at 2, 4, 6 months, clinical examination, evaluation of possible manifestation of allergy in the child until the end of follow-up.

2.3.1. Anthropometric measurements:

- **Weight** - children's weight was measured with an electronic baby scale Seca 354, in kilograms with an accuracy of 0.005 kg after removing clothes and diapers;
- **Height** - direct measurement of height was done with a baby height meter SECA 210, in centimeters with an accuracy of 0.1 cm;

• **Head circumference** - with a non-stretchable soft meter SECA 211, passing in front of glabella, laterally - above the ears, and behind through protuberantia occipitalis externa in centimeters to the nearest 0.1 cm .

2.3.2. Scoring through SCORAD (SCORing Atopic Dermatitis) - in case of skin allergy - SCORAD is a clinical tool for assessing the extent and severity of eczema. It defines:

The degree of severity - the parts affected by eczema are marked on a body diagram, determining the percentage of the affected area "A" in relation to the whole body. The percentages of all rash areas are calculated. The total area is 'A', which has a possible value of 100% maximum.

The intensity of rashes in a certain area is assessed as: absent - 0, mild -1 or severe - 2. Redness, swelling, itching/scabs, traces of scratching, thickening (lichenification) and dryness are noted (assessed in areas where there is no inflammation). The scores are added up and the total is "B" (maximum 18).

Subjective symptoms – itching and insomnia. Each is rated by the parent/caregiver using a scale where 0 is no itching or no insomnia and 10 is the worst itching or insomnia. These values are added together to give "C" (maximum value 20).

2.3.3. Evaluation by CoMiSS (Cow Milk's Related Allergy Symptom Score) – in suspected cow milk's protein allergy (CMPA).

2.4. Statistical methods

The following statistical methods were applied:

2.4.1. Descriptive methods

- Frequent analysis on qualitative variables (nominal and ordinal) – calculation on absolute and relative frequencies (%). The results are presented in a table species;
- Variational analysis on quantitative variables - calculation on average magnitude (average arithmetic value, median), default standard deviation, standard mistake on the average arithmetic value, 95% confidence interval.
- *Methods for checking on hypotheses*

1) Parametric methods

- Student's t-test - for comparison on average arithmetic values from 2 independent samples;

- Non-parametric methods

- Pearson's χ^2 test – for analysis on categorical variables magnitudes;

- Method of Kolmogorov-Smirnov for checking for normality on the distribution on quantitative variable;

- Method of Mann-Whitney - for comparing on the average ones between two independent groups.

3) Logistical regression analysis - the logistic one regression analysis is applied for establishment on dependency between the studied dependent and independent variables. This one analysis has also been used for evaluation on ratio on the chances for the factors included in the study. Through the first one stage on the analysis it was calculated the relative item risk for each

variable separately, and through the second – formation on model from statistically significant independent factors (multifactor regression analysis)

The statistical analysis of the data was performed using the software package SPSS 25.0, Jamovi 2.2.2.

2.5. Graphical method

The results of the study are presented in tabular (simple and complex multidimensional tables) and graphical (line, pie-sector and bar charts) form.

The study was approved by Ethics Committee of Medical Research of the Medical University of Varna "Prof. Dr. P. Stoyanov" with Protocols No. 60/23.02.2017 and No. 91/ 21.02.2020.

VI. RESULTS AND DISCUSSION

VI.1. Results

VI.1.1. Socio-demographic characteristic of the participants in the study

120 children at high risk for allergy and their parents were included in the study.

Table 1. General characteristics of the study participants

	Number / average value	**% / standard off _**
Gender		
Female	54	45.0
Male	66	55.0
Gestational age (in weeks)	38.8	±1.08
Number children in the family		
One kid	57	47.5
Two children	57	47.5
Three children	6	5.0
They visit manger childish place	56	46.7
Age of the mother (years)	30.59	±4.53
Education on the mother		
University degree	77	64.2

Secondary school	40	33.3
College	3	2.5
Smoking at the mother	64	53.3
Age of the father in years (years)	33.48	±4.62
Education on the father		
University degree	51	42.5
Secondary school	59	49.2
Primary education	2	1.7
College	8	6.7
Smoking at the father	52	43.3
Ethnic affiliation		
Bulgarian	109	90.8
Turkish	7	5.8
Mixed	3	2.5
Another	1	0.8
Having pets	30	25.0
Urban place of residence	116	96.7

55% from the children included in the study are males (n=66) and 45% (n= 54) - females.

The mean age of the children is 24.01 ±3.87 months (range 13 to 31 months), with the mean gestational age at birth 38.8 ±1.08 gestational weeks. Almost half of the children (46.7%, n =56) attend a daycare center at the time of the study.

The distribution of children, according to the environment of upbringing, shows a preponderance of covered children with a high risk of allergy, raised in an urban environment - 96.7% (n =116) compared to 3.3% (n =4) of children raised in a rural environment.

Children with the largest relative share are of Bulgarian ethnicity - 90.8% (n=109), followed by 5.8% (n=7) of Turkish origin, 2.5% (n = 3) of mixed origin and other origins (foreign citizen) - 0.9% (n =1).

Every second child (55.2%) - 55.6% of girls and 56.1% of boys has brothers or sisters, and 44.8% of the examined population do not have (44.4% of girls and 43.9% of boys). Less than half of the children in the study sample are born vaginally (49.2 %; n =59), and 50.8% (n = 61) were born by Caesarean section (46.3% of girls (n =25) and 54.5% from the boys (n =36).

According to the method of conception, 94.1% (n =113) of the sample were conceived naturally, a 5.9% (n =7) - through assisted reproduction, of which 5% (n =6) - through in vitro fertilization and 0.9% (n =1) - through insemination.

The data analysis reported a mean age for the mothers of 30.59 ± 4.53 years (range 21÷42) years and 33.48 ± 4.62 years (rank 22÷44) years for the fathers.

Regarding the "education" indicator, the mothers of the children from the studied group have a higher educational level, compared to the fathers - 64.2% (n =77) of mothers have a higher education, compared to 42.5% (n =51) of fathers. 49.2% (n =59) of the fathers and 33.3% (n =40) of the mothers have secondary education.

The children included in the study have the characteristics of children from the general population - with educated parents, urban residence and Bulgarian ethnicity.

V.1.2. Allergic manifestations

About half of the mothers - 56.7% (n=68) of the examined children had manifestations of allergy, compared to 43.3% (n = 52) who did not report allergic manifestations. The opposite ratio was found in the group of fathers: 45.8% (n =55) of the fathers had manifestations of allergy, and 54.2% (n =65) didn't have. The lowest frequency of allergic manifestations of the other family members was found in the siblings - one third 33.3% (n =40), while the majority – (66.7%, n =80) did not have any (Figure 1).

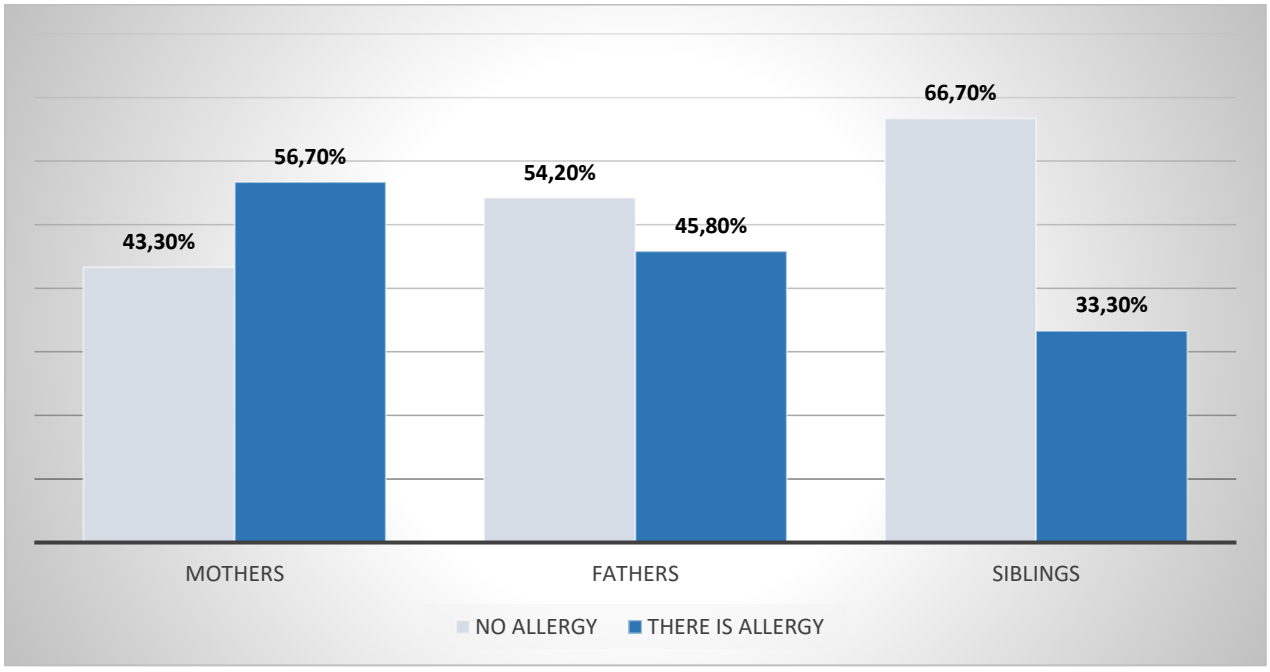

Figure 1. Structural distribution of allergic manifestations in other family members (parents and siblings)

The data analysis shows that in 72.2% (n =39) of the girls in the studied population, there is no family history of atopic dermatitis (AD), and in 27.8% (n =15) there is. For males, the results were 71.2% (n =47) and 28.8% (n =19), respectively. On average, 71.7% (n =86) of the examined children had no family history of AD, and 28.3% (n =34) had family history of AD (Figure 2).

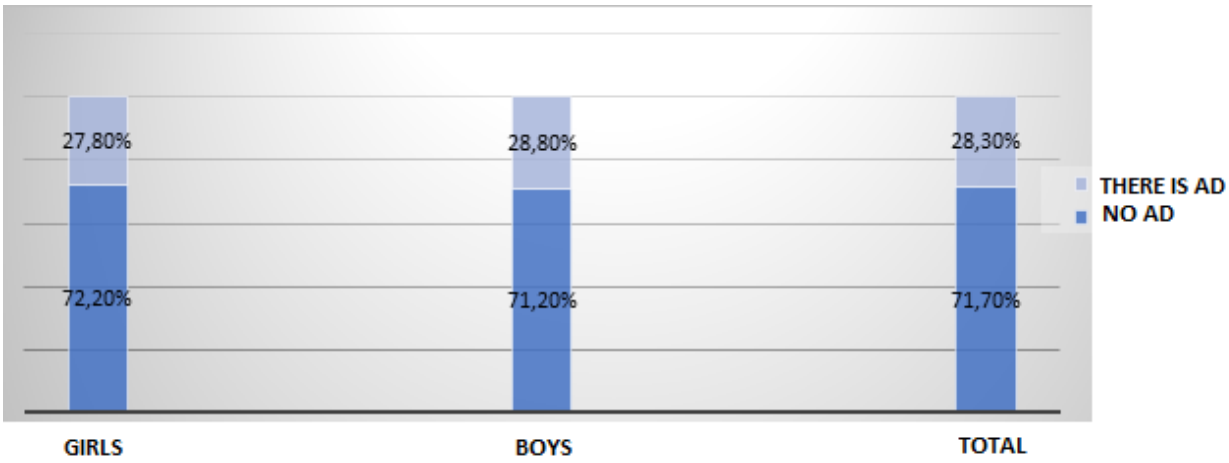

Figure2. Structural distribution of the studied children with family history of atopic dermatitis

Boys predominate statistically - 55.9% (n =19) versus 44.1% (n =15) girls ($p < 0.05$) among the children with family history for AD. Cases of AD in the family were reported in about 1/3 of the children in the study - 28.3% (n =34). Table 2 shows the frequency of allergic manifestations and their type in the family members of the sampled children.

Table 2. Frequency of allergic manifestations in the families of the examined children

Kind of allergy	**Mother (n;%)**		**Father (n;%)**		**Siblings (n;%)**	
Allergies	68	56.7	55	45.8	40	33.3
Asthma	26	21.7	25	20.8	10	8.3
Nasoconjunctivitis	42	35.0	38	31.7	15	12.5
Atopic dermatitis	7	5.8	4	3.3	20	16.7
Urticaria	12	10.0	13	10.8	5	4.2
Food allergies	18	15.0	8	6.7	8	6.7

A very weak negative correlation was found between the type of feeding from birth and the type of allergic manifestation, which is not statistically significant ($p>0.05$). Only in asthma the correlation is weakly positive, but again without statistical significance ($r =0.17$; $p=0.06$), (Table 3).

Table 3. Frequency of allergic manifestations in the examined children according to the way of feeding from birth on

Type of allergy	**Way of feeding from birth on (n; %)**			Total	χ^2; r	Pearson r; p
	breastfeeding	mixed	substitute			
Allergies total	17 (44.7)	26 (43.3)	7 (31.8)	50 (41.7)	1.09;0.58	-0.08; 0.38
Asthma	0	2 (3.3)	2 (9.1)	4 (3.3)	3.57; 0.17	**0.17; 0.06**
Nasoconjunctivitis	1 (2.6)	1 (1.7)	0	2 (1.7)	0.59; 0.75	-0.07; 0.46
Atopic dermatitis	8 (21.1)	7 (11.7)	1(4.5)	16(13.3)	3.57; 0.17	-0.17; 0.61
Urticaria	4 (10.5)	7 (11.7)	2 (9.1)	13(10.8)	0.12; 0.94	-0.01; 0.91
Food allergy	4 (10.5)	11 (18.3)	0	15(12.5)	5.15; 0.76	-0.07; 0.43
Non-medicated allergy	5 (13.2)	3 (5.0)	2 (9.1)	10 (8.3)	2.05; 0.36	-0.07; 0.42

V.1.3. Predisposing factors for allergy manifestations

Only a quarter of children with a family history of allergy - 25% (n =30) are raised in an environment with a pet. The distribution by gender shows a similar frequency: 25.9% (n =14) of girls and 24.2% (n =16) of boys have a pet.

Every second child is raised by smoking parents - 53.3% (n =64). Distributed by gender – the parents of 53.7% (n =29) of the girls and 53.0% (n =35) of the boys were smokers.

Analyzing the maternal smoking factor during pregnancy, it was found that 13.3% (n =16) of the pregnant women smoked, and 86.7% (n =104) did not smoke, with the gender distribution of the studied contingent of children having a slight preponderance of mothers of girls – 14.8% (n =8), compared to boys – 12.1% (n =8) - (Figure 3).

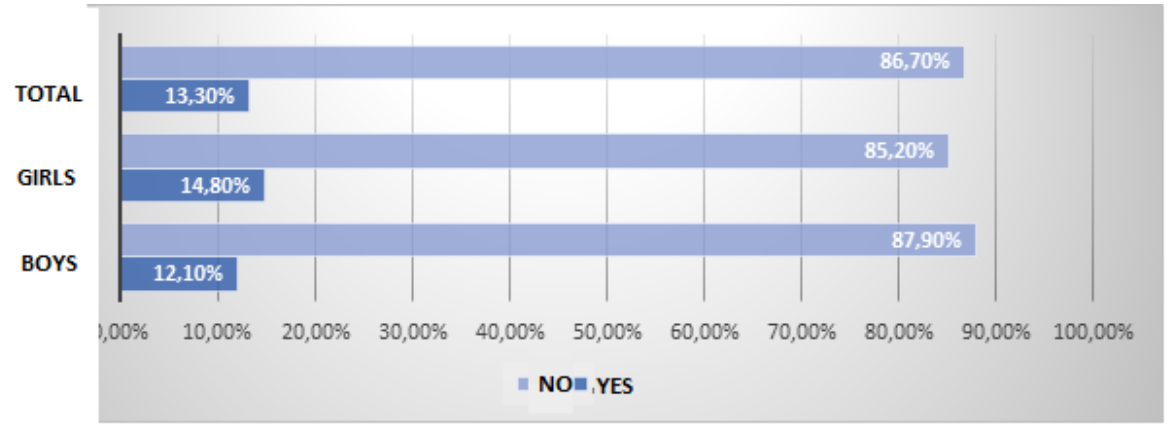

Figure 3. Structural distribution of children according to maternal smoking during pregnancy

The gender distribution of children of smoking mothers was found to be 50% (n =8) to 50% n =8). Statistical analysis shows that 43.3% (n =52) of the fathers of the examined children are smokers, and 56.7% (n =68) are non-smokers. Fathers' smoking was almost equally common among boys and girls, as with smoking mothers: 42.6% (n =23) of girls' fathers were smokers and 43.9% (n =29) of boys' fathers (Figure 4).

55.8% (n =29) of the group of smoking fathers were of boys, and 44.2% (n =23) - of girls.

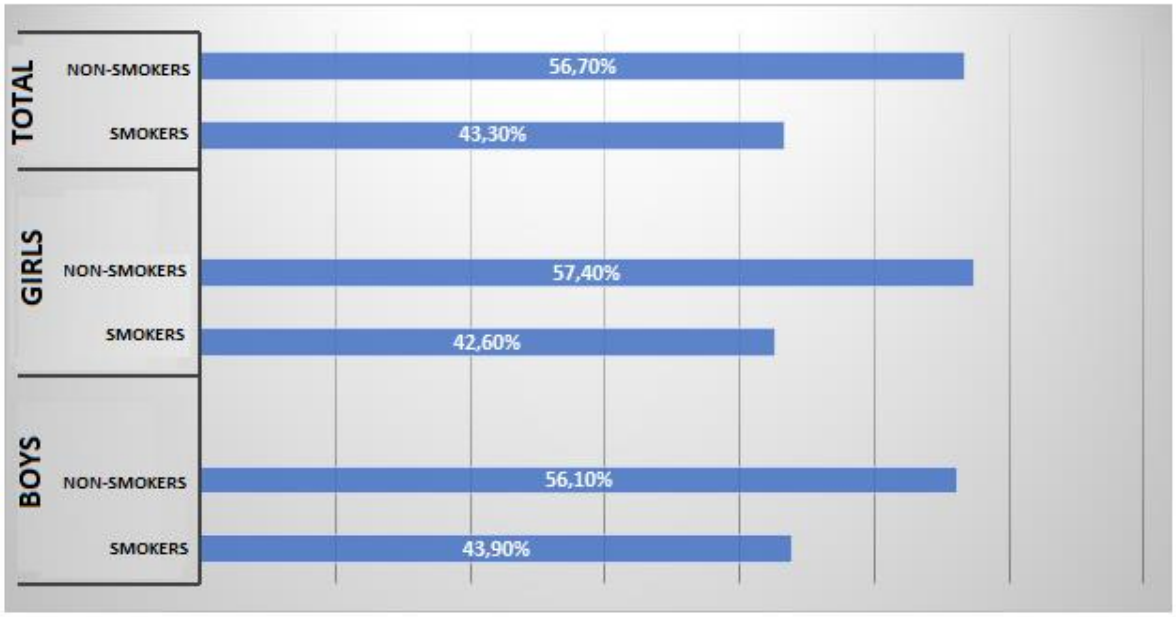

Figure 4. Structural distribution of the examined children according to the father's smoking

V1.1.4. Effect of breastfeeding on the growth of children with family history of allergy

The growth of children with a family history of allergy included in the study was assessed based on anthropometric indices and WHO standards - height-for-age (HA), weight-for-age (WA), weight-for-height (WH), index for body mass-for-age (IBMA) according to the discriminatory criteria and standards of the World Health Organization (WHO, 2006/2007) for assessing growth and development in healthy children.

We found in our study that the average weight was lower in absolute value in breast-fed children, compared to children on replacement/mixed feeding only at birth: 3325.3±328.96 g versus 3395.8±410.35 g, while for the measurements at the 2-nd, 4-th and 6-th months the weight has higher values, although at statistically insignificant levels ($p>0.05$).

The analysis of the anthropometric indices, according to the age of the examined children (at the age of 1 and at the age of 2) depending on the method of feeding - breastfed or on substitute/mixed, indicates that the method of feeding is important for the growth of children. Children on substitute/mixed feeding have faster rate of weight gain at 2 years of age with statistical significance in the parameters WH (0.02±1.25) and (0.65±1.21), ($p =0.01$), WA (0.26±1.08) and (0.74±0.86), ($p=0.01$) and IBMA (2 years) – (-0.02±1.31) and (0.64±1.32), ($p=0.02$) - (Table 4).

Table 4. Comparative analysis of the anthropometric indexes adjusted to the children's age depending on the type of feeding - at 1 year and 2 years of age(Unpaired samples Student 's _ t - test)

		Breastfed		Substitute + mixed		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
WH (1 year)	-0.02	0.53	1.46	0.54	1.35	0.98
HA (1 year)	0.09	0.88	1.06	0.86	1.17	0.93
WA (1 year)	-0.03	0.77	1.05	0.78	1.02	0.98
IBMA (1 year)	-0.04	0.38	1.58	0.39	1.46	0.97
WH (2 years)	-2.54	0.02	1.25	0.65	1.21	**0.01**
HA (2 years)	-0.29	0.40	1.29	0.48	1.23	0.77
WA (2 years)	-2.56	0.26	1.08	0.74	0.86	**0.01**
IBMA (2 years)	-2.47	-0.02	1.31	0.64	1.32	**0.02**

The analysis of the deviations in the anthropometric indexes, according to the age of the studied children at the age of 2 years, shows that the type of milk that the child took during the breastfeeding period has borderline statistical significance ($p = 0.05$) for the indicators WH, WA and IBMA at 2 years of age (breastfeeding – (-0.02±1.31), (0.02±1.25) and (0.26±1.08), standard

infant formula (IF) (0.66±1.42), (0.65±1.31) and (0.69±0.94) or hydrolyzed IF – (0.60±1.18), (0.63±1.09) and (0.81±0.73). The children from the sample, fed with IF (in mixed or substitute way of nutrition) have a faster pace of weight gain at 2 years of age, especially those fed with standard IF (Table 5).

Table 5. Analysis of variance of age-adjusted anthropometric indices in 2-year-old children depending on the type of milk during the breastfeeding period

		Breast milk		Standard milk		Hydrolysate milk		
	F	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
TR (2 years)	3.05	-0.02	1.31	0.66	1.42	0.60	1.18	**0.05**
RV (2 years)	0.63	0.40	1.29	0.36	1.32	0.64	1.09	0.54
TV (2 years)	3.11	0.02	1.25	0.65	1.31	0.63	1.09	**0.05**
ITMV (2 years)	3.09	0.26	1.08	0.69	0.94	0.81	0.73	**0.05**

Examining the relationships between the type of nutrition and the anthropometric indicators of growth in the studied group of children with a family history of allergy, a positive weak and statistically significant correlation was found between the type of milk the child received and WH at the age of 2 (rho = 0.20; p=0.034), as well as between the type of milk and IBMA at 2 years of age (rho = 0.20; p=0.035) - (Appendix 1).

Analyzing the anthropometric growth indicators of the examined children up to 2 years of age with/without manifestation of AD, it becomes clear that the indicators: HA at 2 years -0.54±1.17 and -0.11±1.54, respectively (p=0.05) and WA at 2 years 0.68±0.94 and 0.09±0.84, (p =0.018) were statistically significantly higher in children without AD compared to the indicators of children with AD (Table 6).

Table 6. Comparative analysis of the anthropometric indicators of growth of children from the sample up to 2 years of age with and without manifestation of atopic dermatitis

		Without atopic dermatitis		**With atopic dermatitis**		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
Weight 2 months (g)	0.36	5224.44	555.97	5163.33	518.03	0.72
Height - 2 months(cm)	0.98	57.49	2.17	56.83	2.25	0.33

Weight 4 months (g)	0.59	6731.46	744.25	6587.00	627.43	0.56
Height 4 month(cm)	0.17	63.33	2.40	63.20	2.04	0.87
Weight 6 month(g)	0.18	7824.55	931.68	7767.78	629.32	0.86
Height 6 months(cm)	-0.39	67.76	2.56	68.11	2.26	0.70
WH (1 year)	-0.60	0.51	1.39	0.73	1.29	0.55
HA (1 year)	0.40	0.88	1.13	0.76	1.22	0.70
WA (1 year)	-0.41	0.76	1.03	0.87	1.00	0.68
IBMA (1 year)	-0.63	0.36	1.50	0.61	1.41	0.54
WH (2 years)	1.04	0.51	1.27	0.16	1.14	0.30
HA (2 years)	1.98	0.54	1.17	-0.11	1.54	**0.050**
WA (2 years)	2.40	0.68	0.94	0.09	0.84	**0.018**
IBMA (2 years)	0.78	0.49	1.35	0.20	1.31	0.44

VI.1.4.1. Effect of breastfeeding on the growth of boys, born with family history of allergy

Conducting the anthropometric studies at the age of 2, 4 and 6 months of the examined boys, it is noticeable that the average weight of the breastfed boys is lower of absolute value, compared to the children on substitute/mixed nutrition only at birth: 3395.3±250.08g and 3481.8±404.27g, while for the measurements at the 2-nd, 4-th and 6-th months of age, the weight has higher values, although at statistically insignificant levels ($p>0.05$).

When tracking the anthropometric indicators WH, HA, WA and IBMA at 1 and 2-years of age, depending on the type of nutrition, we find that when the studied indicators increase with age, the type of nutrition has no statistical significance in boys, but still all growth indicators are higher in children on substitute/mixed nutrition.

The analysis of deviations in anthropometric indicators adjusted for age in boys at 2 years of age shows that the type of milk (breast milk, standard or hydrolyzed IF), by which the child was fed during infancy, does not have any statistical significance.

Studying the relations between the type of nutrition and anthropometric indicators in boys, it was found:

- A positive moderate and statistically significant correlation between the duration of breastfeeding in general (presence of breastmilk in the nutrition of the boys) and weight at 4 months of age ($r =0.41$; $p=0.01$);
- Positive moderate and statistically significant correlation between duration of breastfeeding and weight at 6 months of age ($r =0.34$; $p=0.04$);

- Negative moderate and statistically significant correlation dependence between the type of milk during infancy and the weight at 4 months of age (r = - 0.39; p=0.01);
- A positive moderate and statistically significant correlation between the type of nutrition (breastfeeding or mixed/substitution) during infancy and WH at 1 year of age (r =0.28; p=0.03), as well as with IBMA at 1 year of age (r = 0.29; p=0.02) - (Appendix 2).

Analyzing the anthropometric growth indicators of boys up to 2 years of age with/without manifestation of AD, we find that the indicators: length at 2 months - 58.43±2.7 cm and 56.50±2.27 cm (p =0.021) , weight at 4 months - 7103.95±674.02 g and 6523.33±335.18 g (p =0.046) and TV at 2 years 0.84±1.2 and 0.12±0.68 (p =0.024) are lower in children with AD and have statistical significance, and the indicators of weight at 2 months of age – respectively 5514.17±477.38 g and 4981.25±436.92 g, (p =0.005) and WH at 2 years – 0.857±1.39 and 0.0442±0.82, (p = 0.056) – have borderline statistical significance (Table 7) .

Table 7. Comparative analysis of anthropometric growth indicators of sampled boys up to 2 years of age with and without atopic dermatitis

		Without atopic dermatitis		With atopic dermatitis		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
Weight at 2 m (cm)	2.93	5514.17	477.38	4981.25	436.92	**0.005**
Height per 2m (cm)	2.38	58.43	2.7	56.50	2.27	**0.021**
Weight at 4m (cm)	2.06	7103.95	674.02	6523.33	335.18	**0.046**
Height at 4 m (cm)	0.36	64.18	2.29	63.83	1.94	0.72
Weight at 6 m (cm)	1.33	8247.22	882.56	7698.00	671.25	0.19
Height at 6 m (cm)	0.01	68.61	2.58	68.60	2.70	0.99
TR (1 year)	-1.03	0.23	1.54	0.73	1.43	0.30
RV (1 year)	0.39	0.59	1.3	0.46	1.16	0.69
TV (1 year)	-0.87	0.44	1.12	0.75	1.12	0.38
ITMV (1 year)	-1.04	0.11	1.67	0.66	1.55	0.30
TR (2 years)	1.94	0.86	1.39	0.04	0.82	**0.056**
RV (2 years)	0.61	0.40	1.26	0.14	1.61	0.54
TV (2 years)	2.31	0.84	1.2	0.12	0.68	**0.024**
ITMV (2 years)	1.71	0.83	1.49	0.06	1.03	0.09

VI.1.4.2. Effect of breastfeeding on the growth of girls born with family history of allergy

Conducting the anthropometric measurements at the age of 2, 4 and 6 months of the examined girls, it is obvious that the average weight of the girls has a higher absolute value in the exclusively breastfed girls, compared to the girls on replacement/mixed feeding as in birth, as well as in measurements in all indicated periods, although at statistically insignificant levels ($p>0.05$). The height of the studied group of girls was however slightly greater in the children on substitute/mixed feeding, again at statistically insignificant levels ($p>0.05$).

Tracking the anthropometric indicators WH, HA, WA and IBMA at the age of 1 and at the age of 2 years, depending on the type of nutrition of the girls, we find that when the studied indicators increase with age, the type of nutrition has statistical significance for the indicator WA (2 years), $p=0.007$. Girls on substitute and mixed feeding have a greater weight at 2 years of age than breastfed (Table 8).

Table 8. Comparative analysis of the average values of the indicators WH, HA, WA and IBMA at 1 year and 2 years in girls depending on the type of nutrition (Unpaired samples Student's _t - test)

		Breastfeeding		Substitute +mixed		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
WH (1 year)	1.68	1.14	1.14	0.62	1.07	0.10
HA (1 year)	-0.76	1.06	1.06	1.31	1.19	0.45
WA (1 year)	1.32	1 .00	0.74	1.01	0.78	0.19
IBMA (1 year)	1.70	0.99	1.26	0.41	1.18	0.10
WH (2 years)	-1.63	-0.15	1.07	0.35	1.06	0.10
HA (2 years)	-1.86	0.20	1.33	0.79	0.97	0.07
WA (2 years)	-2.79	0.05	0.92	0.75	0.76	**0.007**
IBMA (2 years)	-1.40	-0.14	1.16	0.31	1.13	0.17

The analysis of age-adjusted anthropometric indicators in 2-year-old girls shows that the type of milk that the child received during infancy (breast milk, standard or hydrolyzed IF), has statistical significance only for the indicator WA at 2 years of age. The highest rate of weight gain was found in girls who were fed by standard IF 0.76±0.89 g compared to those who were fed by hydrolyzed IF 0.65 ±0.61 g and breastfed 0.055±0.92 g, ($p=0.045$), (Table 9).

Table 9. Analysis of variance of deviations in age-adjusted anthropometric indicators in 2-year-old girls depending on the type of milk during the breastfeeding period

		Breast milk		Standard IF		Hydrolyzed IF		
	F	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
WH (2 years)	1.34	-0.15	1.07	0.34	1.23	0.35	0.87	0.28
HA (2 years)	1.50	0.21	1.33	0.90	1.10	0.68	0.82	0.24
WA (2 years)	3.40	0.05	0.92	0.76	0.89	0.65	0.61	**0.045**
IBMA (2 years)	0.99	-0.14	1.16	0.31	1.32	0.32	0.96	0.38

Examining the relations between the type of nutrition and the anthropometric indicators of girls, it was found:

- A negative moderate and statistically significant correlation between the type of milk, received by the female children and weight at 4 months of age (r =- 0.39; p=0.012);
- A positive moderate and statistically significant correlation between the duration of breastfeeding as a general (presence of breastmilk in child's nutrition) and WH at 1 year (r =0.282; p=0.047), as well as IBMA at 1 year (r =0.304; p=0.032);
- A positive moderate and statistically significant correlation between the duration of breastfeeding and WH at 1 year (r = 0.334 ; p=0.018), as well as IBMA at 1 year (r = 0.357; p=0.011);
- A positive moderate and statistically significant correlation between the type of feeding (substitute/mixed) during infancy and WH at 1 year of age (r =0.28; p=0.028), as well as with IBMA at 1 year of age (r =0.29; p=0.023), (Appendix 3).

The weight at the age of 2 months of the girls with/without manifestation of AD is lower in the subgroup without AD, compared to those with AD - respectively 4912.44±460.61g and 5527.50±522.77g (p =0.016), but the WA indicator of 2 years - respectively 0.7±1.06 and -0.85±1.22, (p=0.007) is statistically significantly higher in children without AD compared to the indicator of children with pronounced AD (Table 10).

Table 10. Comparative analysis of anthropometric indicators of growth of girls from the sample up to 2 years of age with and without the appearance of atopic dermatitis

		Without atopic dermatitis		With atopic dermatitis		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
Weight-2 months	-2.52	4912.44	460.61	5527.50	522.77	**0.016**
Length at 2 months	-1.04	56.487	1.81	57.50	2.380	0.30
Weight at 4 months	-1.10	6315.15	586.54	6682.50	986.66	0.27
Length at 4 months	0.12	62.38	2.19	62.25	2.06	0.91
Weight at 6 months	-1.35	7333.71	733.19	7855.00	661.14	0.19
Length at 6 months	-0.64	66.78	2.19	67.50	1.73	0.53
WH (1 year)	0.13	0.81	1.14	0.74	0.92	0.89
HA (1 year)	-0.78	1.19	1.15	1.75 _	1.03	0.44
WA (1 year)	-0.34	1.10	0.80	1 .00	0.40	0.73
IBMA(1 year)	0.24	0.62	1.25	0.47	1.07	0.80
WH (2 years)	-0.67	0.14	1.01	0.52	1.97	0.50
HA (2 years)	2.80	0.70	1.06	-0.85	1.22	**0.007**
WA (2 years)	1.21	0.52	0.83	-0.03	1.34	0.23
IBM (2 years)	-0.90	0.11	1.07	0.65	2.09	0.37

In the regression model for the influence of the factors: smoking of the parents, AD in the family, mode of birth, duration of breastfeeding, breastfeeding in the first days after birth, a statistically significant influence was found mainly of AD in the family on the WA indicator at 2 years ($p = 0.021$, Adjusted $R^2 = 0.16$).

VI.1.5. Effect of breastfeeding on acute morbidity of children with a family history of allergy

VI.1.5.1. Effect of breastfeeding on acute morbidity of boys with a family history of allergy

Examining the acute morbidity in the boys from the sample with/without food allergy, we found that children with FA suffered more often from acute nasopharyngitis 5.00 ± 5.27 times versus 2.05 ± 2.19 times, (p =0.004). Also, the total duration of acute nasopharyngitis during the follow-up period was longer in the male children with FA - 37.44 ± 42.12 days, compared to the duration in male children without FA 13.25 ± 15.82 days, (p =0.002). The results have statistical significance.

The number of acute gastroenteritis suffered by the boys with FA - 1.00 ± 1.41 is also statistically higher than that of the boys without FA - 0.33 ± 0.66 (p = 0.023). The number of hospitalizations due to acute gastroenteritis was also statistically higher in boys with FA manifestation - 0.33 ± 0.71, compared to the number of hospitalizations in boys from the sample without FA manifestation - 0.052 ± 0.23 , (p=0.020).

In general, the total number of acute infections suffered by boys with FA - 7.78 ± 6.53 - was higher than that of boys without FA - 4.14 ± 4.67, although without significant statistical significance (p=0.056) - (Table 11).

Table 11. Comparative analysis of the number and duration of acute illnesses of boys with/without food allergy

		With food allergy		**Without food allergy**		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
General number infections	-1.95	7.78	6.534	4.14 _	4.668	**0.056**
Respiratory infections-number	-1.65	6.33	6.442	3 .14	4.367	0.10
Nasopharyngitis-number	-2.97	5.00	5.268	2.05	2.191	**0.004**
Bronchiolitis-number	-0.18	1.22	1.302	1.05	2.715	0.85
Laryngitis-number	0.96	0.00	0.000	0.16	0.492	0.34
Acute pneumonias-number	0.27	0.22	0.667	0.28	0.590	0.78
Acute gastroenteritis-number	-2.33	1.00	1.414	0.33	0.664	**0.023**
Acute urinary infections-number	0.56	0.00	0.000	0.04	0.186	0.57
Other infections-number	0.23	0.22	0.441	0.30	0.981	0.82

General duration on the infections-days	-1.34	55.33	49.447	34.56	42.328	0.18
Respiratory infections-duration-days	-1.35	50.56	48.136	30.58	40.095	0.18
Acute nasopharyngitis duration - days	-3.21	37.44	42.119	13.25	15.816	**0.002**
Acute laryngitis duration-days	0.95	0.00 _	0.00	1.12	3.511	0.34
Acute urinary infections duration-days	0.53	0.00	0.00	0.79	4.41	0.59
Number of hospitalizations	0.08	0.44	0.88	0.47	1.02	0.93
Hospitalizations for respiratory infections	0.84	0.11	0.33	0.37	0.90	0.40
Hospitalizations for gastroenteritis	-2.39	0.33	0.71	0.05	0.23	**0.020**
Hospitalizations for urinary infections	0.39	0.00	0.00	0.02	0.13	0.69

Analyzing the relationship between the acute morbidity of the boys from the sample and the way of nutrition, the type of milk they receive, nutrition at birth (with/without breast milk), we find that there is a negative moderate and statistically significant correlation between the total number of cases of acute nasopharyngitis and the way of feeding – exclusively breastfed boys suffer less often from acute nasopharyngitis (r=-0.26), (p=0.04). A negative moderate and statistically significant correlation was also found between the total duration of acute nasopharyngitis and feeding from birth with breast milk (r =-0.26), (p =0.048) - (Appendix 4 and Appendix 5).

V1.5.2. Effect of breastfeeding on acute morbidity in girls with a family history of allergy

Examining the acute morbidity of the girls from the sample with/without manifestation of food allergy, we find that the number of acute bronchiolitis in girls with FA is significantly more - 1.00±1.27 than in girls without manifestation of FA - 0.27±0.676, with statistical significance (p =0.030). Also, the duration of illnesses from acute laryngitis is longer as an absolute value in children with HA - 3.33±5.16 days, compared to the duration in children without HA - 0.96±2.47 days, despite the lack of statistical significance (p =0.059).

In general, the total number of hospitalizations of girls with FA manifestation (0.67±0.82 hospitalizations) was higher than that of girls without FA (0.35±1.12) hospitalizations and has statistical significance (p= 0.030) - (Table 12).

Table 12. Comparative analysis of mean number and duration of acute illnesses in girls with/without food allergy

		With food allergy		Without food allergy		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
General number of infections	-0.41	4.33	4.97	3.19	2.95	0.69
Respiratory infections-number	-0.81	3.50	4.81	2.17	2.40	0.42
Nasopharyngitis-number	-0.16	2.00	3.95	1.33	2.26	0.88
Bronchiolitis-number	-2.24	1.00	1.26	0.27	0.68	**0.03**
Laryngitis-number	-1.09	0.67	1.21	0.27	0.79	0.28
Acute pneumonias-number	0.47	0.00	0.0 0	0.125	0.64	0.64
Acute gastroenteritis-number	-1.02	0.67	0.82	0.29	0.85	0.31
Acute urinary infections-number	0.57	0.00	0.00	0.15	0.62	0.57
Other infections-number	0.30	0.17	0.41	0.25	0.67	0.77
General duration on the infections (days)	-0.53	31.83	30.88	25.83	25.46	0.60
Respiratory infections-duration-(days)	-0.88	26.33	32.19	18.20	19.76	0.38
Acute nasopharyngitis duration (days)	-0.19	13.67	27.76	12.92	16.18	0.85
Acute bronchiolitis duration (days)	-1.56	9.33	12.99	3.15	8.66	0.13
Acute laryngitis duration (days)	-1.93	3.33	5.16	0.96	2.47	0.06
Acute pneumonia duration (days)	0.54	0.00	0.00	2.19	9.78	0.59
Acute gastroenteritis duration (days)	-1.78	4.33	5.89	1.50	3.56	0.69
Acute urinary infections duration (days)	0.52	0.00	0.00	2.83	13.73	0.42
Others infections duration	0.39	1.17	2.86	2.04	5.41	0.88
Number of hospitalizations	-0.66	0.67	0.82	0.35	1.12	**0.030**
Hospitalizations for respiratory infections	-0.67	0.50	0.84	0.25	0.86	0.28
Hospitalizations for gastroenteritis	-0.73	0.17	0.41	0.063	0.32	0.64
Hospitalizations for urinary infections	0.50	4.33	4.97	0.042	0.20	0.31

Analyzing the relationship between the acute morbidity of the girls from the sample and the type of nutrition, the type of milk they receive, nutrition at birth (with/without breast milk), we find that there is a moderate negative and statistically significant correlation between the total number of hospitalizations due to diseases of the respiratory system and the duration of breastfeeding in months (r =-0.309), (p=0.033). A moderate negative and statistically significant correlation was also demonstrated between the type of milk received by girls from the sample and the number of hospitalizations due to diseases of the digestive system (r =-0.311), (p =0.031) – (Appendix 6, Appendix 7).

V.6. Influence of breastfeeding on allergy manifestation of children with a family history of allergy

120 children with family history of allergy were included in our study, 41.7% (n =50) of which had a clinical manifestation of various forms of allergy during the observation and the manifestation was more frequent in males: 43.9% (n =29) of the boys and 38.9% (n =21) of the girls (Figure 5).

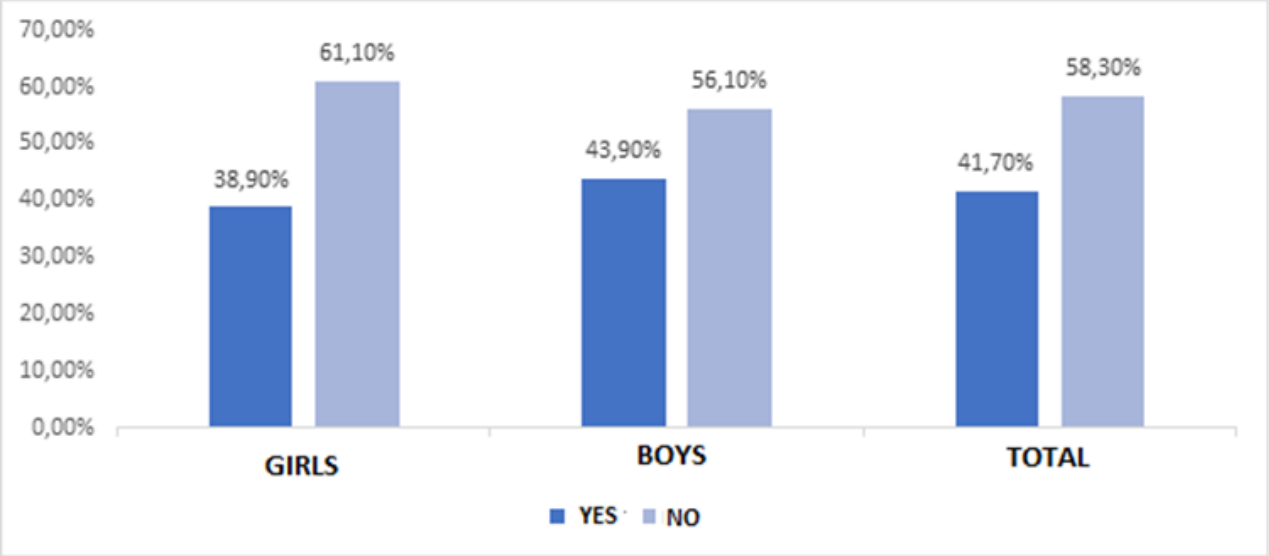

Figure 5. Relative share of examined children who showed clinical allergy

Regarding the manifestation of the different forms of allergy according to the gender of the child, it was found that the most common manifestation in the female gender is urticaria - at 14.8% (n =8), and in the male - AD - at 18.2% (n = 12) of the cases, despite the lack of statistical significance regarding the clinical manifestation of the different forms of allergy in girls and boys (p >0.05), (Table 13).

Table 13. Manifestation of allergy in different genders

	Female n (%)	Male gender n (%)	p
Total allergies	21 (38.9%)	29 (43.9%)	0.57
Asthma	1 (1.9 %)	4 (4.5%)	0.41
Allergic nasoconjunctivitis	1 (1.9%)	1 (1.5%)	0.88
Atopic dermatitis	4 (7.4%)	12 (18.2%)	0.08
Urticaria	8 (14.8%)	5 (7.6%)	0.20
Food allergy	6 (11.1%)	9 (13.6%)	0.67
Drug allergy	5 (9.3%)	5 (7.6%)	0.74

Table 14 shows the manifestation of different types of allergies according to the type of milk nutrition during the first months of life. Allergies occurred most often in 44% (n =22) of children fed with standard milk. A statistical relationship was established for food allergy and the intake of standard IF (p =0.02), where 66.7% (n =10) of the children fed by standard MK manifested HA. In the other forms of allergy, no statistical dependence was established p >0.05).

Table 14. Manifestation of types of allergy according to the type of milk feeding in the first months of life

	Total allergies n (%)	Asthma n (%)	AllergicNC n (%)	Atopic dermatitis n (%)	Urticaria n (%)	Food allergy n (%)
Breast milk	14 (28%)	0 (0%)	1 (50%)	7 (43.8%)	4 (30.8%)	5 (33.3%)
Hydrolyzed IF	14 (28%)	3 (75%)	1 (50%)	3 (18.8%)	4 (30.8%)	0 (0%)
Standard IF	**22(44%)**	1(25%)	0 (0%)	6 (37.5%)	5 (38.5%)	10 (66.7%)
p-value	0.892	0.120	0.483	0.301	0.966	**0.02**

According to the duration of exclusive breastfeeding during the first months of life, the most allergic manifestations are found in children fed with breast milk for less than 6 months - 76% (n =38) of those who reported allergy, followed by those exclusively breastfed for more than 12 months – 20% (n =10) and the least – in those exclusively breastfed between 6 and 12 months, despite the lack of statistical significance (p >0.05). Statistical significance was established in the occurrence of allergic nasoconjunctivitis (NC) and the duration of exclusive breastfeeding – no child breastfed for more than 12 months showed allergic NC (p =0.025). For the other forms of allergy, statistical significance was not established (Table 15).

Table 15. Manifestation of types of allergy according to the duration of exclusive breastfeeding during the first months of life

Duration of exclusive breastfeeding	Total allergies n (%)	Asthma n (%)	Allergic nasoconjunctivitis n (%)	Atopic dermatitis n (%)	Urticaria n (%)	Food allergy n (%)
< 6 months	**38 (76%)**	4 (100%)	1 (50%)	12 (75%)	10 (76.9%)	13 (86.7%)
6 -12 months	2 (4%)	0 (0%)	1 (50%)	1 (6.3%)	1 (7.7%)	0 (0%)
> 12 months	10 (20%)	0 (0%)	0 (0%)	3 (18.8%)	2 (15.4%)	2 (13.3%)
p-value	0.688	0.456	**0.025**	0.954	0.822	0.363

According to the duration of the presence of breast milk in the diet during the first months of life, the most allergies were found in children who were breastfed for less than 6 months - in 66% (n =33) of those who showed different allergic manifestations, despite the lack of statistical significance (p>0.05). This was not found in the different forms of allergy and the duration of breastfeeding during the first months of life (p >0.05) - (Table 16).

Table 16. Manifestation of types of allergy according to the duration of the presence of breast milk in the diet during the first months of life

Duration of presence of breastmilk in the diet	Total allergies n (%)	Asthma n (%)	Allergic nasoconjunctivitis n (%)	Atopic dermatitis n (%)	Urticaria n (%)	Food allergy n (%)
Breastfeeding < 6 months	**33(66%)**	4(100%)	1 (50%)	9 (56.3%)	9 (69.2%)	11 (73.3%)
Breastfeeding 6 -12 months	5 (10%)	0 (0%)	1 (50%)	3 (18.8%)	1 (7.7%)	1 (6.7%)
Breastfeeding > 12 months	12 (24%)	0 (0%)	0 (0%)	4 (25%)	3 (23.1%)	3 (20%)
p-value	0.977	0.328	0.147	0.442	0.933	0.761

Table 17 shows the manifestation of different types of allergies according to the type of nutrition during the first months of life. We found that as a total number, more allergies were manifested in those who received IF - at 72% (n=36), while in those who were breastfed it was 28% (n=14) of all children with allergic manifestations. In all types of allergy the relative share of

those fed mixed/substitutional is greater compared to breastfed, with the exception of allergic NC, where the ratio is 1:1? Statistical significance was not found (p >0.05).

Table 17. Manifestation of the types of allergy according to the type of nutrition in the first months of life

	Total allergies n (%)	**Asthma** n (%)	**Allergic naso-conjunctivitis** n (%)	**Atopic dermatitis** n (%)	**Urticaria** n (%)	**Food allergy** n (%)
Breastfeeding	14 (28%)	0 (0%)	1(50%)	7(43.8%)	4(30.8%)	5(33.3%)
Mixed + substitute	**36 (72%)**	4(100%)	1(50%)	9(56.3%)	9(69.2%)	10(66.7%)
p-value	0.945	0.201	0.493	0.142	0.836	0.646

The relationship between the way of feeding immediately after birth and the manifestation of allergies is shown on Table 18. The most cases of allergies are in the group of children who have been on a mixed diet since birth - 26 (52%) of all who reported an allergy. In general, with the exception of AD, in all other nosological units, the largest number of children who showed an allergy were from the mixed-fed group. No statistical significance was found between the type of feeding (breastfeeding, mixed, substitute) and the appearance of allergies in the studied group of children (p>0.05).

Table 18. Manifestation of the types of allergy according to the way of feeding from birth

Nutrition from birth on	**Total allergies** n (%)	**Asthma** n (%)	**Allergic RC** n (%)	**Atopic dermatitis** n (%)	**Urticaria** n (%)	**Food allergy** n (%)
Breast feeding	17 (34%)	0 (0%)	1 (50%)	8 (50%)	4 (30.8%)	4 (26.7%)
Mixed	**26 (52%)**	2(50%)	1 (50%)	7 (43.8%)	7 (53.8%)	11 (73.3%)
Substitute	7 (14%)	2(50%)	0 (0%)	1 (6.3%)	2 (15.4%)	0 (0%)
p-value	0.579	0.167	0.745	0.167	0.944	0.076

VI.7. Influence of breastfeeding on ND in children with family history of allergy
VI.7.1. Influence of breastfeeding on ND in boys with family history of allergy

Comparing the mean scores in the individual domains of ND of the boys from the sample depending on the smoking status of the mothers, we find lower values in the boys whose mothers are smokers. A statistically significant difference in scores was found for all areas, except for physical development (Table 19).

Table 19. Comparison of the neurodevelopment of boys according to the status of maternal smoking (Independent Samples t – test)

		Smoking mother		**Mother non-smoker**		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
Physical development	1.74	88,90	13,28	96.90	18,20	0.09
Adaptive behavior	2.54	89.40	14.69	100.90	17.40	**0.01**
Socio - emotional development	2.29	84.60	11.27	93.60	15.60	**0.03**
Cognitive development	2.72	94.10	9.68	103.80	14.30	**0.01**
Communication	2.00	93.80	14.58	103.00	17.70	**0.05**
Total development	2.57	86.50	11.55	98.00	18.10	**0.01**

How the presence of other siblings and their number affects the ND of boys from the sample is shown on Table 20. The highest scores in all areas are achieved by boys who have one brother/sister. A statistically significant difference in grades was found only for cognitive development ($p=0.001$), while for the other areas the differences found were not statistically significant ($p \geq 0.05$).

Table 20. Comparison of the neurodevelopment of boys according to the presence of siblings in the family (Analysis of variance)

		No others siblings		One sibling		Two others siblings		
	F	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
Physical development	2.02	90.30	11.66	98.80	19.76	95.70	28,37	0.20
Socio-emotional development	2.76	94.50	14.47	101.60	20.15	90,90	5.31	0.09
Cognitive development	9.62	90.70	9.49	93.30	18.97	76.50	5.84	**0.001**
Adaptive behavior	0.83	99.50	10.67	103.20	16.55	95.60	11.51	0.47
Communication	0.39	98.60	14.90	102.50	20.00	97.70	13,26	0.69
General development	1.07	92.00	13.08	98.10	20.64	89.10	14.55	0.39

The number of family members has an impact on the ND of boys and the highest scores in all areas were achieved by those boys who were raised in a family of four (have 1 sibling). A statistically significant difference in evaluations was found only for physical development (p=0.03) and social-emotional development (p=0.01), (Table 21).

Table 21. Comparison of neurodevelopment of boys according to the number of family members (Analysis of Variance)

		3-member		4-member		5-member		6-member	family	
	F	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
Physical development	5.06	86.20	8.61	99.50	21.43	99.30	12.67	99.80	32.95	**0.03**
Adaptive behavior	1.98	91.90	13.94	102.70	21.59	99.70	13.87	92.10	6.23	0.17

Socio - emotional development	5.50	87.70	9.36	95.20	20.43	91.60	11.91	81.00	2.92	**0.01**
Cognitive development	1.10	97.90	9.82	105.70	17.11	98.80	12.94	99.50	11.03	0.40
Communication	1.17	95.50	13.09	104.80	20,61	101.80	17.02	97.40	17,22	0.37
General development	2.09	88.50	11.33	99.70	21.88	97.30	14.75	91.10	16.89	0.17

The duration of breastfeeding correlates very weakly and statistically insignificant with the assessment of ND of boys (as a general development and in different areas) when controlling factors like smoking in the house, number of siblings, number of family members, age of the father and smoking of the mother ($p \geq 0.05$).

In the regression model for the influence of the factors smoking in the house, number of siblings, duration of exclusive breastfeeding and the presence of breast milk in the child's diet, the type of feeding at birth, only the use of IF versus breast milk were the statistically significant factors on the total ND of boys ($p=0.02$ for hydrolyzed IF and $p=0.01$ for standard IF).

VI.7.2. Effect of breastfeeding on the ND of girls with family history of allergy

Examining the ND of the girls from the sample and the influence of breastfeeding on it, we find higher values in the female children of mothers who don't smoke. A statistically significant difference in scores was found for the following areas: physical development ($p=0.04$), adaptive behavior ($p=0.03$), cognitive development ($p=0.04$) as well as general development ($p=0.04$), (Table 22).

Table 22. Comparison of ND of girls according to the status of maternal smoking

		Smoking mother		**Mother - non-smoker**		
	Student's t	**Mean**	**SD**	**Mean**	**SD**	**p**
Physical development	1.74	88,90	13,28	96.90	18,20	0.09
Adaptive behavior	2.54	89.40	14.69	100.90	17.40	**0.01**
Socio – emotional development	2.29	84.60	11.27	93.60	15.60	**0.03**

Cognitive development	2.72	94.10	9.68	103.80	14.30	**0.01**
Communication	2.00	93.80	14.58	103.00	17.70	**0.05**
General development	2.57	86.50	11.55	98.00	18.10	**0.01**

Regarding the influence of the siblings on the ND of the girls from the sample, we find that the highest scores in all areas except physical development were achieved by those girls who had no siblings. Statistically significant differences in the scores were found for socio-emotional development (p=0.01) and general development (p<0.001), (Table 23).

Table 23. Comparison of neurodevelopment of girls according to the presence of siblings in the family (Analysis of variance)

		No sibling		One sibling		Two siblings		
	F	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
Physical development	5.19	89.10	12.25	90.60	11.79	79.00	3.88	0.07
Socio - emotional development	18.61	81.90	15.38	81.40	15.81	51.60	5.71	**0.01**
General development	31.02	89.60	14.93	87.60	17.25	66.90	2.24	**< .001**

We find that the number of family members also influences the ND of the girls from the sample and the highest scores in physical development and adaptive behavior were achieved in those girls, living in a family of five, while the highest scores in socio-emotional and cognitive development, in communication and general development have girls raised in families of four (they have 1 more brother/sister). A statistically significant difference in scores was found for communication (p =0.03) and for the general development (p =0.01), (Table 24).

Table 24. Comparison of the neurodevelopment of girls according to the number of family members (Analysis of Variance)

		3-member		4-member		5-member		6-member	family	
	F	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**

Socio – emotional development	3.18	78.10	16. 34	84.60	13.27	80.60	22.23	55.60	11.41	0.13
Communication	6.39	91.40	20.82	**98.80**	17.70	89.70	18.69	68.20	7.42	**0.03**
Total development	8.20	85.80	17.40	**90.80**	14.82	88.50	17.66	68.40	4.48	**0.01**

The type of milk, the type of feeding (breastfeeding/substitute) and the feeding at birth very weakly and statistically insignificant correlated with the assessment of ND of girls, as the general development and other areas, when controlling for the factors smoking in the house, number of siblings, number of family members, age of the father and smoking of the mother ($p \geq 0.05$).

There is no statistical significance of the influence of each of the factors: smoking in the house, number of siblings, duration of exclusive breastfeeding and the presence of breast milk in the child's diet, type of feeding at birth, use of IF versus breast milk, maternal smoking in the regression model for their influence on the general ND of the girls with family history of allergy ($p>0.05$).

In the regression model for the influence of the factors: smoking at home, number of siblings, duration of exclusive breastfeeding and the presence of breast milk in the child's diet, type of feeding at birth, use of infant formula versus breast milk, maternal smoking, statistically missing significant influence of each of the factors on the total ND of the girls ($p >0.05$).

VI.2. Discussion

In order to access the health status of children who are at risk of allergy, it is extremely important to study their family history, to obtain information about the perinatal and postnatal period, the way of nutrition, the type of milk nutrition, the time of introducing solids, the type, severity, duration of acute diseases, of a possible manifestation of allergy, as well as to carry out a thorough physical examination together with anthropometric measurements, in order to obtain data about the real health status of children, on their growth and ND.

VI.2.1. Breastfeeding and growth

Growth assessment of children with family history for allergy was based on anthropometric indexes and WHO standards, according to WHO (2006, 2007) discriminative criteria and norms for assessment of growth and development in healthy children. The WHO growth curves use the growth pattern of breast-fed children as their standard and are applied to monitor and evaluate growth. Infant and young child feeding practices should aim for weight gain consistent with generally accepted WHO standards.

The relationship of allergic diseases with growth disorders – weight loss and suppression of skeletal maturation was first documented by *Cohen* in 1940 who proposed monitoring of the growth of children with allergic diseases *(Cohen MB. et al., 1940).*

Breastfeeding has been inversely associated with weight gain and height during infancy in multiple observational and prospective studies (*Kalies et al., 2005*). An earlier peak in infant BMI was also observed with longer duration of exclusive breastfeeding. The longer exclusive and partial breastfeeding is associated with a slower rate of growth during infancy *(Patro-Gołąb et al., 2019b*). This association was more pronounced with exclusive than with partial breastfeeding, which matches with our results. We find that the average weight of the studied group has a lower absolute value in exclusively breastfed children, compared to children on replacement/mixed feeding only at birth: 3325.3±328.96 g (for breastfed) and 3395.8±410.35 g (for replacement and mixed fed), while at the measurements of the 2nd, 4th and 6th months the weight has higher values, although at statistically insignificant levels ($p>0.05$). Analyzing the changes of the anthropometric indices, according to the age of the examined children at the age of 2 years and depending on the way of feeding, it was found that the children on substitute/mixed feeding have a faster growth rate at the age of 2 years with statistical significance of results for the WH, WA and IBMA ($p<0.05$). In various meta-analyses, rapid weight gain in infancy and in the second year of life above average norms defined by growth reference standards has been consistently associated with an increased subsequent risk of obesity *(Brands B. et al, 2014), (Druet C et al., 2012).* Both - the higher birth weight and the rapid rate of weight gain during the first 2 years of life have been found to be associated with an increased risk of obesity *(Koletzko et al., 2009).* In addition to the higher risk of obesity, higher weight at age 2 is associated with various later-onset pathological conditions, such as increased risk of hypertension, increased body fat deposition, worse lipoprotein profile, and diabetes *(Brands et al., 2014).*

Rapid weight gain in the first 2 years of life is associated with twice higher risk of school-age asthma, and especially rapid weight gain in the first 3 months is associated with an increased risk of asthma (*Rzehak et al., 2013b).*

In a prospective cohort study including 1928 children born in Boston, *Tsai* and co-authors *(Tsai et al., 2018b)* found that children with a rapid rate of weight gain during the first 2 years of life and who were overweight were at risk of developing asthma in later childhood.

Accelerated weight gain can lead to increased deposition of adipose tissue in the thorax and abdomen, which can reduce pulmonary vital capacity and thus increase obstruction-related pulmonary resistance and the risk of asthma *(Permaul et al., 2014).* Adipose-related inflammation and the effect of the energy-regulating hormones leptin and adiponectin can induce a tissue-specific immunological and inflammatory effect with remodeling of lung and airway tissue as well *(den Dekker et al., 2017).*

Higher concentrations of adiponectin in breast milk are associated with significantly lower weight during the first 6 months of life in breastfed infants. This undoubtedly makes adiponectin an early regulator of weight in the neonatal period and in infancy. It could serve as a biomarker for other bioactive factors in breast milk that affect weight gain too. Therefore, breast milk

components can influence weight gain *(Grunewald et al., 2014).* Although rarely, exclusive breastfeeding can also cause excessive weight gain. In some cases, this has been observed in association with a higher content of produced protein in breast milk than would normally be expected *(Grunewald et al., 2014), (Prentice et al., 2016).* Overall, breastfeeding is associated with a modest reduction in the risk of overweight and obesity in later childhood. Complementary foods should not be introduced before the age of 17 weeks and no later than 26 weeks. Associations between higher protein intake in early childhood and overweight or obesity in later life have been reported in many studies *(Weijs et al., 2011), (Koletzko et al., 2019).*

Compared to breast-feeding, feeding by standard infant formulas induces higher average weight gain during the first year of life and beyond *(Koletzko et al., 2019).* Our results are also similar, showing that the children fed by standard IFs have the fastest weight gain, followed by those fed with hydrolyzed IFs, and the most physiological weight gain have those fed with breast milk. The analysis of the deviations of the anthropometric indicators, according to the age of the examined children at the age of 2 years, shows that the type of milk that the child took during infancy has a borderline statistical significance ($p=0.05$) in the indexes WH, WA and IBMA at 2 years of age. According to our results, children fed by infant formula (in mixed and replacement feeding) had a faster rate of weight gain at 2 years of age, especially those fed by standard IF. We establish a positive weak and statistically significant correlation between the type of milk the child received and WH at 2 years of age ($r=0.20$; $p=0.034$), as well as between the type of milk and IBMA at the age of 2 ($r=0.20$; $p =0.035$).

Breastfeeding should be encouraged and supported. In addition to many other benefits, it can contribute to reducing the risk of later overweight and obesity. Predominant and partial breastfeeding should also be encouraged if exclusive breastfeeding is not achieved. Breastfeeding is associated with many benefits and is recommended as the preferred method of infant feeding *(Prell and Koletzko, 2016), (Agostoni et al., 2009).*

Children with atopic dermatitis have slower growth rate than healthy children. This is most often explained by the food restriction. It is defined as restriction of three or more types of food due to AD and HA and is associated with a lower Z-score for weight, height, head circumference, BMI. Acute illnesses are an independent risk factor for dietary restriction. The most frequently restricted foods are seafood, nuts, eggs, cow's milk, and dairy products. The earlier the atopic dermatitis debut is, the more difficult the course is. In children who have food restrictions related to atopic dermatitis and food allergy, there is a lower intake of calories, proteins, carbohydrates, fats, riboflavin, vitamin B_{12}, phosphorus, calcium and iron, negatively affecting their growth. Severity of AD is an independent risk factor affecting weight and height. Nutritional intake in children with AD should be systematically assessed and parents should be regularly counseled regarding nutrient intake, especially in children with moderate or severe AD *(Low et al., 2020b).* In our study, analyzing the anthropometric indicators of growth of the examined children up to 2 years of age without/with the manifestation of AD, we find that the values of the HA indexes at 2 years - respectively (0.54 ± 1.17) and (-0.11 ± 1.54), ($p =0.05$) and WA at 2 years (0.68 ± 0.94) and

(0.09±0.84), (p=0.018) were higher in children without AD compared to children with pronounced AD and had statistical significance.

The data of our study coincide with the data of the literature that, in general, children suffering from AD have a slower growth rate than their healthy peers *(Agostoni et al., 2000).* Children's growth and development best reflect their health status. It has been proven that AD has an adverse effect on the growth of the child's organism *(Ip S. et al., 2007).*

Weight and height percentiles, Z-scores, and growth velocity of children with AD are lower than those of healthy children *(Owen et al., 2005).* Skin inflammation leads to altered cytokine/chemokine production and causes systemic inflammation. The systemic mechanism of AD also affects systemic metabolism *(Yamamoto-Hanada et al., 2021).* The degree of growth disturbance correlates with the severity of AD – children with a mild course of AD are less affected compared to those with severe disease *(Ip S et al., 2007), (Yamamoto-Hanada et al., 2021).* In addition to dietary restrictions, there are many other factors that contribute to affect the growth of children with AD, such as increased metabolism due to rapid skin renewal, sleep disturbances, and loss of nutrients through the skin *(Low et al., 2020b).* The literature indicates other causes that can slow the growth of children with atopy, such as local growth factors:

- PgE2 (Prostaglandin E2) - an important factor in bone maturation, which is also involved as a mediator in allergic reactions of the fast and delayed type),
- PAF (Platelet activating factor) – one of the most powerful mediators in the pathogenesis of allergic reactions, which affects the synthesis of PgE2 in osteoblasts
- Chronic stress and growth hormone dysregulation *(Baum et al., 2002).*

These relationships indicate that atopy-dependent disturbances in the complex system of local and systemic growth factors could lead to a disturbance in skeletal maturation, which in turn leads to a delay in growth and development in children suffering from AD. When analyzing the anthropometric indicators of growth of boys up to 2 years of age without/with the manifestation of AD, we find that the results for length at 2 months - 58.43±2.7cm and 56.50±2.27cm, p=0.021, weight at 4 months 7103.95±674.02 g and 6523.33±335.18 g, p=0.046 and WA at 2 years 0.84±1.2 and 0.12±0.68 are lower in children with AD and the difference has statistical significance, and the indicators of weight at 2 months – respectively 5514.17±477.4 g and 4981.25 ±436.93 years, p=0.005 and WH at 2 years - 0.86±1.39 and 0.04±0.82, p=0.056 - have borderline statistically significant difference.

In the examined female children up to 2 years of age without/with AD, the weight at 2 months was lower in girls without AD, compared to those with AD - respectively (4912.44±460.61 g) and (5527.50±522.77 g), p=0.016, but the HA index at 2 years - respectively (0.70±1.06) and (-0.85±1.22), (p=0.007) was higher in girls without AD compared to that of girls with pronounced AD at statistical significance of the results.

Girls have a relatively more severe course of AD than boys and therefore have lower values of all growth parameters, especially height, as we found in the children we observed. As early as 1987, *Kristmundsdottir F and David TJ* reported that skeletal retardation was more common in girls with AD *(Kristmundsdottir and David, 1987).*

VI.2.2. Breastfeeding and acute morbidity

Breastfeeding alters the child's gut microbiome and subsequent immune development and influences the risk of respiratory infections through maternal antibody transfer *(Matheson et al., 2012).*

Atopic disorders represent an important health problem. Infections of the upper respiratory tract, middle ear, asthma and AD are the most common diseases in pediatric pathology. There is not much data in the literature regarding the comorbidity of children with allergic diseases. According to many studies, some comorbidities can even influence the course of allergic diseases. For example, upper respiratory tract infections, especially in early childhood are associated with atopic disorders later in life *(Munblit et al., 2017a).* Acute viral "non-RSV bronchiolitis" in children under 6 months of age has been associated with the development of asthma *(Lumia et al., 2012).* A child's developing immune system can be affected by frequent and severe middle ear infections, resulting in asthma and AD *(MacIntyre and Heinrich, 2012b), (Kim et al., 2021).* On the other hand, effusion in the middle ear is associated with the development of allergic rhinitis *(Norhafizah et al., 2020).* According to Munblit *(Munblit et al., 2017b),* children with AD are at increased risk of developing other skin diseases, children with asthma are at risk of other respiratory tract diseases, children with atopic rhinitis are at increased risk of ENT diseases.

Gastrointestinal and musculoskeletal disorders occur more frequently in children with atopic disorders. Children with asthma suffer more often from acute laryngitis, tracheitis, acute respiratory infections, pneumonia and bronchitis. The explanation for this is that respiratory tract infections aggravate asthma symptoms or vice versa. Children with gastrointestinal symptoms are also often diagnosed with atopic disorders. These gastrointestinal symptoms may relate to IgE-mediated food allergies or, rarely, to eosinophilic esophagitis, which is associated with atopic disorders *(Pols et al., 2017).*

According to the data of our study regarding the acute morbidity of the boys from the sample with/without food allergy, we find that male children with FA suffer more often from acute nasopharyngitis (5.00±5.27) compared to those without FA (2.05±2.19), (p=0.004). Also, duration of acute nasopharyngitis has longer duration in boys with FA (37.44±42.12), compared to duration in boys without FA (13.24±15.82), (p=0.002). The results have statistical significance. We find that children with FA suffer from a greater number of acute gastroenteritis and have a greater number of hospitalizations due to acute gastroenteritis. Overall, the total number of acute infections was higher in the FA group, although without statistical significance.

We find in boys a weak and statistically significant correlation between the total number of cases of acute nasopharyngitis and the way of feeding (breastfed get sick less often). There is also a negative weak and statistically significant correlation between the total duration of acute nasopharyngitis and the duration of breastfeeding from birth.

Lower dyspeptic gastrointestinal symptoms such as diarrhea and abdominal pain are common in allergic diseases such as asthma and AD. Although food allergies and rare organic

gastroenterological diseases such as eosinophilic gastroenteropathy are associated with atopic disease, it is unusual for them to explain such symptoms by themselves. Small intestinal biopsies from patients with asthma and allergic rhinitis show features common to the inflammatory response seen in the airways, with accumulation of eosinophils, T cells, mast cells, macrophages, and increased expression of proallergic cytokines, such as IL-4 and IL- 5. Bronchial mucosal inflammation in asthma may influence nasal mucosal inflammation in rhinitis and there is increasing evidence for intestinal mucosal inflammation and altered permeability in patients with allergic disease *(Powell et al., 2007).*

We find that the number of acute gastroenteritis suffered by boys with food allergy (1.00±1.41) was also statistically higher than that of boys without FA (0.33±0.66), (p=0.023). The number of hospitalizations for acute GE was also statistically higher in boys with FA (0.33±0.71) compared to the number of hospitalizations in boys from the sample without FA (0.05±0.22), (p=0.020).

Overall, the total number of acute infections suffered by boys with food allergy (7.78±6.53) was higher than that of boys without food allergy (4.14±4.67), although without significant statistical significance (p=0.056).

Examining the acute morbidity in the girls from the sample with/without manifestation of food allergy, we find that the number of acute bronchiolitis diseases in girls with food allergy is significantly more (1.00±1.26) than in those without manifestation of food allergy (0.27±0.68), at statistical significance (p=0.030). Also, the duration of acute laryngitis was greater in absolute value in children with food allergy (3.33±5.16), compared to the duration in children without food allergy (0.96±2.47), despite the lack of statistical significance (p=0.059).

Overall, the total number of hospitalizations of girls with food allergy in the first 2 years of life (0.67±0.82) was higher than that of girls without food allergy (0.35±1.12) at statistical significance (p=0.030).

Analyzing the relationship between the acute morbidity of the girls from the sample and the way of feeding, the type of milk they receive, feeding at birth (with vs. without breast milk) we find that there is a moderate negative and statistically significant correlation between the total number of hospitalizations due to diseases of the respiratory system and the duration of breastfeeding in months (r=-0.31), (p=0.03). A moderate negative and statistically significant correlation was also demonstrated between the type of milk received by the girls from the studied group of children and the number of hospitalizations due to diseases of the digestive system (r=-0.31), (p=0.031). Infection with respiratory RNA viruses appears to trigger an immune response that has similarities to atopic disease, as both can lead to the production of serum IgE. Furthermore, clinically apparent infection with these viruses appears to correlate with increased atopic sensitization and allergic disease in humans *(Martorano and Grayson, 2018).*

Tian et al., following up children who suffered from *RSV*-bronchiolitis, found that lack of breastfeeding, exposure to tobacco smoke and the deficiency of vitamin A and vitamin D are significant risk factors contributing to bronchiolitis caused by *RSV*. The exposure to tobacco smoke, the deficiency of vitamin A and D, personal and family history of atopy are among the

significant risk factors contributing to post-bronchiolitis wheezing in children with recurrent *RSV* bronchiolitis *(Tian M. et al., 2009).*

Children with a history of other atopic diseases, such as allergic rhinitis and AD, are more likely to develop asthma *(Edwards, L.R. and Borger, J., 2019).*

An increased frequency of otitis media was found in 32.5%, with 46.2% of recurrent infections in a 6-months follow-up of children under 2 years of age who were hospitalized for *RSV* infection *(Kafetzis et al., 2003).* This shows the relationship between otitis media and *RSV* infection. An association between recurrent episodes of wheezing and food allergy has also been established. HA was found in 27.5% of cases *(Nateghian et al., 2017).*

Cow milk's allergy is among the most common allergies in early childhood with an incidence in developed countries of 0.5% to 3% at the age of 1 year *(Flom and Sicherer, 2019).* There are limited data on the incidence and severity of infections in children with cow milk's allergy, although some studies in children with cow milk's allergy have reported a higher incidence and severity of respiratory infections. Children with atopic predisposition have a delayed maturation of the Th1-response through childhood, which increases the risk of infections in the first years of life.

Susceptibility to infections has been well studied in the development of asthma, but there is relatively limited data in the literature regarding susceptibility to infection in food allergy. However, a retrospective study found that sensitization to β-lactoglobulin (cow's milk protein) was associated with an almost fourfold increased risk of recurrent respiratory tract infections in children under two years of age *(Woicka-Kolejwa et al., 2016).* Cow milk's allergy in infancy has also been shown to be associated with recurrent otitis media *(Pazdro-Zastawny et al., 2019).*

VI.2.3. Breastfeeding and allergy expression

There is conflicting evidence regarding the protective role of breastfeeding in relation to the development of allergic sensitization and allergic disease. Studies on this issue differ in methodology and outcome measures, resulting in considerable heterogeneity. The composition of human milk also varies between individuals, which may partially explain the conflicting data. The composition of breast milk is very complex and contains variable levels of immunoactive molecules, oligosaccharides, metabolites, vitamins and other nutrients and different microbial content. Existing evidence suggests that modulation of breast milk composition has the potential to prevent allergic diseases early in life. Breast milk should be the main source of nutrition during the critical period of metabolic and immune programming due to its effects on intestinal function. It has been suggested that the wide range of bioactive factors such as proteins, PUFAs, oligosaccharides, microorganisms, metabolites and trace elements present in breast milk can influence the infant's immune intestinal maturation. Chronic allergic diseases are associated with altered functioning of the innate and adaptive immune system, and evidence suggests that it can be influenced by various interventions. Many studies have shown that various maternal exposures, such as immunizations, diet, vitamin D, omega-3 fatty acids, and/or probiotics, can affect the composition of breast milk and thereby affect the health of the infant *(Munblit et al., 2017b).* The

composition of breast milk varies between postpartum periods and among women, which may partly explain some of the conflicting results of observational studies on breastfeeding. It has been suggested that the conflicting results may be partly due to variation in the composition of breast milk, as it contains a wide variety of immunoactive substances and components that are present in different concentrations *(Agarwal et al., 2011).* In assessing the relationship between breastfeeding duration and health outcomes, it is important to have clear definitions of breastfeeding duration. It is usually defined as the total duration of breastfeeding, which is the time between birth and complete cessation of breastfeeding; while exclusive breastfeeding duration is the time between birth and the first introduction of food other than breast milk.

41.7% (n=50) from 120 children included in the study had a clinical manifestation of various forms of allergy during the observation, and the manifestation was more frequent in boys, compared with the girls - in 43.9% (n=29) from the boys and in 38.9 % (n=21) from the girls.

Regarding the various forms of allergy, it was found that the most common manifestation in females is urticaria - in 14.8% (n=8), and in males - AD - in 18.2% (n=12) of cases, despite the lack of statistical significance regarding the clinical manifestation of different forms of allergy in girls and boys ($p>0.05$). Allergies occurred most often in 44% (n=22) of children fed by standard IF. A statistical dependence was established for FA and the intake of standard IF ($p=0.02$), where 66.7% (n=10) of the children fed by standard IF manifested FA. In the other forms of allergy, no statistical dependence was established ($p>0.05$).

According to the duration of exclusive breastfeeding in the first months of life, the most allergic manifestations are found in children breastfed for less than 6 months - 76% (n=38) of all who reported allergy, followed by those breastfed for more than 12 months - 20% (n=10) and least – in those who were breastfed between 6 and 12 months, despite the lack of statistical significance ($p>0.05$). Statistical significance was found in the occurrence of allergic nasoconjunctivitis and the duration of breastfeeding - no child, fed for more than 12 months, showed allergic nasoconjunctivitis ($p=0.025$). Statistical significance was not established for the other forms of allergy.

According to the duration of breastfeeding in general, the most allergies were manifested in children who were breastfed for less than 6 months - in 66% (n=33) of those who showed various allergic manifestations, despite the lack of statistical significance ($p>0.05$). This was not found in the different forms of allergy and the duration of breastfeeding in the first months of life ($p>0.05$).

As a total number, more allergies were manifested in those who received IF - at 72% (n=36), while in those who were breastfed it was 28% (n=14) of all children with allergic manifestations. It is noteworthy that for all nosological units, the relative share of those fed mixed/substitute is greater, compared to exclusively breastfed, with the exception of allergic nasoconjunctivitis, where the ratio is 1:1. Statistical significance was not established ($p>0.05$).

It was found, from all children who had shown some type of allergy, the most were from the group who has been on mixed feeding immediately after birth – 26 (52%). In general, with the exception of AD, in all other nosological units, the largest number of children who showed an allergy were from the mixed-fed group. No statistical significance was found between the type of

feeding (breastfeeding, mixed, replacement) and the appearance of allergies in the studied group of children ($p>0.05$).

Breastfeeding is associated with increased thymus size. The size of the thymus (assessed by ultrasound) in exclusively breastfed infants at 4 months of age was more than twice the size of the thymus of children fed by infant formula - an effect that persisted until at least 10 months of age. Continued breastfeeding between 8 and 10 months also correlated with increased thymus size in a "dose-dependent" manner. Although the immune implications of this remain unclear, a subsequent study showed a correlation between breastfeeding and peripheral CD4 and CD8, T-cell counts and proportions *(Jeppesen et al., 2004).*

Human milk contains many immuno-active factors, including leukocytes (polymorphonuclear neutrophils, monocytes/macrophages, lymphocytes), which can potentially influence the immune status in early childhood. Over 250 potentially immunoactive proteins have been found in its composition, including a wide variety of cytokines, inflammatory mediators, prebiotic oligosaccharides, PUFAs and a diverse microbiome. All of them are involved in complex interactions that can affect immune status. Colostrum is very rich in immunoactive molecules, which are present in much higher concentrations than in mature milk. The levels of growth factors in colostrum decline very rapidly, which may be partially explained by an increase in dilution, as during the first days of life the infant's quantitative needs are low *(Munblit et al., 2016).* As breast milk matures, the relative concentrations of immunologically active molecules decrease with increasing milk volume and the infant's nutritional needs.

VI.2.4. Breastfeeding and neurodevelopment

Along with many positive short-term medical benefits of breastfeeding, a growing body of research suggests that there are also significant long-term ND benefits from breastfeeding *(Bar et al., 2016).* Breastfeeding has clear short-term benefits for the child by reducing morbidity and mortality from infectious diseases. Most studies studying breastfeeding and its long-term impact show that children breastfed for more than 6 months have better cognitive abilities, a lower risk of developing attention deficit hyperactivity disorder (ADHD) and a lower risk of autism spectrum disorders (ASD). There is already much evidence in the literature that early nutrition and lifestyle have long-term effects on later health and morbidity ("metabolic programming").

There are critical periods when the organs and systems of the human body are very plastic and sensitive to the environment. Most influences occur in utero, but the brain, liver, and immune system remain susceptible to influences even after birth. Therefore, the ND of young children is influenced by many factors *(Victora et al., 2015).* The first 2 years of life are a critical period of rapid brain growth and development. During this period, nutrition and environmental factors play an important role in the child's growth and cognitive development *(Nurliyana et al., 2016).* Substantial differences in ND between breastfed and formula-fed children have been reported, but results may be confounded by differences in parental education, intelligence, family environment, and socioeconomic status *(Eidelman, 2012).*

According to some scientists, breastfeeding has little or no effect on children's intelligence. They believe that breastfeeding, despite its many advantages for the child and the mother, hardly leads to an increase in the intelligence of the child, but the intelligence of the mother impacts the child's intelligence more than the breastfeeding itself *(Der et al., 2006).*

We found in our research that the highest scores were achieved in cognitive development in boys of non-smoking mothers - 103.8, in smoking mothers - 94.10; lowest values – in the social-emotional development domain: 93.6 and 84.6 respectively. The comparison of the average scores in the separate areas of the children's ND depending on the smoking status of the mothers shows lower values in the boys whose mothers are smokers. A statistically significant difference in scores was found for all areas, except for physical development: adaptive behavior ($p=0.01$), cognitive ($p=0.01$), social-emotional development ($p=0.03$), as well as general behavior ($p= 0.01$).

The presence of other children in the family and their number have an impact on the NDP, with the highest scores in all domains achieved by boys who have one brother or sister. A statistically significant difference in grades was found only for cognitive development ($p<0.001$), while for the other areas the differences found were not statistically significant ($p\geq0.05$).

The number of family members affects the ND of the boys from the sample, finding that the highest scores in all domains were achieved by those boys who were raised in a family of four (having 1 brother or sister). We find a statistically significant difference in the scores for physical development ($p=0.03$) and social-emotional development ($p=0.01$).

The duration of breastfeeding of boys correlates very weakly and statistically insignificant with the assessment of ND as a general development and in individual areas when controlling for factors such as smoking in the house, number of brothers and sisters, number of family members, age of the father and smoking of the mother ($p \geq0.05$).

In the regression model for the influence of the factors smoking in the house, number of siblings, duration of breastfeeding in total and duration of exclusive breastfeeding, type of feeding at birth, only the use of infant formula versus breast milk were the statistically significant factors on the total ND of boys ($p=0.02$ for hydrolyzed IF and $p=0.01$ for standard IF).

Studying the ND of the girls from the sample and the influence of breastfeeding on it, higher values were found in the children of non-smoking mothers. The highest scores were achieved in the field of cognitive development: 90.00 in children of smoking mothers and 96.6 in children of non-smoking mothers, and the lowest values in general development (81.10 and 90.80 respectively). The comparison of the average scores in the different areas of the ND of the girls depending on the smoking status of the mothers shows lower values in the girls whose mothers are smokers. A statistically significant difference in scores was found for the following areas: physical development ($p=0.04$), adaptive behavior ($p=0.03$), cognitive development ($p=0.04$), as well as general development ($p=0.04$).

Regarding the presence of other children in the family on the ND of girls, it was found that the highest scores in all areas, except physical development, were achieved by those girls who did not have brothers or sisters. Statistically significant differences in scores were found in the areas of socio-emotional development ($p=0.01$) and general development ($p<0.001$).

The number of family members has an effect on girls' ND, finding that the highest scores in physical development and adaptive behavior were achieved by those girls living in a family of five, while the highest scores in socio-emotional and cognitive development, communication and general development have girls raised in families of four. A statistically significant difference in scores was found for communication ($p=0.03$) and general development ($p=0.01$).

In the regression model for the influence of the factors: smoking in the house, number of siblings, duration of breastfeeding and exclusive breastfeeding, type of feeding at birth, use of infant formula versus breast milk, maternal smoking, there is no statistically significant influence of any of the factors on the total ND of girls ($p>0.05$).

Numerous authors have investigated the relationships between infant feeding and cognitive development with conflicting results. Several studies have reported modestly lower IQ scores in formula-fed children compared to breast-fed children, while others have reported no association between infant feeding and intelligence. Observational data should be interpreted with caution due to confounding of data by socioeconomic status and maternal intelligence. Nevertheless, data from 2 randomized controlled trials in Honduras provide evidence of differences in development with different durations of breastfeeding *(Dewey et al., 2001)* - 2 groups were randomized - with the introduction of complementary foods at 4 months and with continued exclusive breastfeeding up to 6 months after birth. Babies in the formula group started crawling later than those who were exclusively breastfed from 4 to 6 months. They were also less likely to walk at 12 months.

Consistent associations between breast-feeding and children's ND have been attributed to breast milk's content of LC-PUFAs for decades. However, the beneficial effect of LC-PUFAs fortification of infant milk on ND remains controversial.

These studies were conducted prior to the introduction of MCs enriched with LC-PUFAs, which were added to IFs to improve neurocognitive outcomes. However, a meta-analysis found that most well-conducted randomized trials showed no benefit of LC-PUFAs over control formula in terms of visual acuity or ND in term infants *(Simmer et al., 2011).* These findings make it unlikely that IFs enriched with LC-PUFAs reduced outcome differences between children in intervention and control groups in these studies *(Stuebe A., 2009).*

Such results were reached in a state-of-the-art large-scale study by French scientists, examining the relationship between the consumption of LC-PUFA-enriched IFs and ND in 9372 children up to 3.5 years of age - ELFE study *(Martinot P. et al., 2022).* They found that the enrichment of IF with LC-PUFA did not lead to an improvement of the ND in the studied cohort of children aged 0 to 3.5 years.

Regarding the possible mechanisms for the effect of breastfeeding on ND, children who were breastfed demonstrated greater gray matter volume in the left and right parietal and left temporal lobes and greater activation in the right frontal and temporal lobes by relation to perceptual tasks, while in language tasks activation was higher in the left temporal lobe. In addition, subcortical gray matter volume has been reported to mediate the relationship between breastfeeding and IQ *(Horta et al., 2018).*

VII. CONCLUSIONS

1. The assessment of the influence of breastfeeding on growth in the first years of life of children with history of allergy confirms the fact that:

a. The way of feeding matters their growth. Breast-fed children have a healthier growth rate, consistent with WHO standards and criteria, while children on replacement/mixed feeding have a faster rate of weight gain at 2 years of age with statistical significance in TP, TV and BMI indicators;

b. The type of milk that the child took during the breastfeeding period has a borderline statistical significance in the WH, WA and IBMA indicators at 2 years of age. Children fed IF (mixed or replacement feeding) had a faster rate of weight gain at 2 years of age, especially those fed standard IF;

c. Children without AD have a faster growth rate in height and weight at 2 years than those with AD, with statistical significance of the results.

1. A. About the boys in the sample:

- All growth indicators are higher in those on replacement/mixed feeding than breastfed, although the type of feeding has no statistical significance for growth;
- The type of milk does not have a statistically significant effect on growth up to 3 years of age;
- The occurrence of AD is a statistically significant factor, adversely affecting growth during the studied period;

1. B. About the girls:

- The type of feeding has statistical significance for growth: the WA indicator at 2 years of age in girls on substitute and mixed feeding is higher than that of breast-fed;
- The manifestation of AD is important for the growth of girls - the indicator TV (2 years) is higher in girls without AD;
- The type of milk that the girls from the sample were fed during infancy is a statistically significant factor for growth - the highest rate of weight gain is found in girls who took standard IF;

2. Conclusions about the acute morbidity of children from the sample:

2. A. About the boys:

- Boys with FA had more frequent acute infections during the study period than those without FA.
- Boys with FA suffer from acute nasopharyngitis more often and for a longer period of time than boys without FA;
- Boys with FA suffer more often from acute gastroenteritis and are hospitalized more often than those without FA;

- Exclusively breastfed boys suffer from acute nasopharyngitis less often, and in boys who are breastfed from birth, the total duration of acute nasopharyngitis illnesses is shorter;

2. B. About the girls:

- Girls with FA appear to suffer from acute bronchiolitis significantly more often than girls without FA;
- Girls with FA suffer longer from acute laryngitis compared to those without FA
- They have a higher total number of hospitalizations for acute infections than those without FA;
- The duration of breastfeeding is important for the number of hospitalizations due to diseases of the respiratory system;
- The type of milk they receive is important for the number of hospitalizations due to diseases of the digestive system - girls receiving breast milk are hospitalized less often due to diseases of the digestive system;

3. Analyzing the manifestations of allergy and their relationship with milk feeding in the studied group of children, the following conclusions are drawn:

a. In male gender, the manifestation of allergies is more common than in female;
b. The most common clinical manifestation in boys is AD, and in girls – urticaria;
c. Allergies occur most often in children fed by standard IF;
d. Children fed by standard IF more often have FA;
e. According to the duration of exclusive breastfeeding and breastfeeding in total, the most allergic manifestations are found in children exclusively breastfed less than 6 months;
f. The duration of breastfeeding is a statistically significant factor for the occurrence of allergic nasoconjunctivitis within the studied period;
g. According to the type of feeding, more allergies occur in children on substitute/mixed feeding than in breastfed

4. Evaluating the ND of children born in families with a history of allergy up to 3 years of age, as well as the influence of breastfeeding on it, we find that:

4. A. About the boys:

a. The number of siblings matters ND - the highest scores in all areas are achieved by those boys who have one sibling.
b. The number of family members has an effect on ND with the highest scores in all domains achieved by those boys raised in families of four.
c. The duration of breastfeeding did not affect the ND score, when controlling for the factors smoking in the house, number of siblings, number of family members, age of father and smoking of mother;

d. The use of infant formula versus breast milk is a statistically significant factor for boys' total ND

e. Boys whose mothers are smokers show lower values in all areas of ND;

4. B. About the girls:

a. Girls whose mothers are smokers show lower values in all areas of ND.

b. Regarding the presence of siblings on the girls' ND, the highest scores in all domains, except physical development, were achieved by those girls who had no brothers or sisters.

c. The number of family members matters girls' ND - the highest scores in physical development and adaptive behavior were achieved by those raised in families of five, while the highest scores in socio-emotional and cognitive development, in communication and general development were achieved by those raised in families of four.

VIII. CONTRIBUTIONS

A. Contributions of original character

For the first time in our country it is being studied:

1. The influence of breastfeeding (as exclusivity and duration, as well as a presence of breast milk in the child's diet in general) on the health (growth, acute morbidity, manifestation of allergies) of children with a family history of allergy in early childhood - up to 3 years;

2. ND of the children from the mentioned sample and the influence on it of the way of feeding and of some epigenetic factors;

3. The influence of the type of milk feeding on growth, acute morbidity, manifestation of allergies and ND of children at risk of allergy;

4. The influence of the duration of breastfeeding on the pointed out indicators in children with family history of allergy.

B. Contributions of a confirmatory nature

Our study confirmed that:

- Children with a family history for allergy fed by IF, especially those with standard IF, have more rapid growth rate;
- Breastfed babies get sick rarely and are less often hospitalized due to diseases of the respiratory and digestive systems as well as having a shorter hospital stay than those fed by IF;
- Children with pronounced AD have a slower growth rate than those without AD;
- Those with FA (in our study – only boys) suffer from acute gastroenteritis more often and for a longer period of time;
- The manifestation of allergies is more common in boys than in girl;
- Allergies occur most often in children fed by standard IF;

- Children with FA suffer more often from acute illnesses and are hospitalized more often;
- Most allergies occur in children breastfed for less than 6 months;
- Children of smoking mothers have lower ND indicators;
- Children (in our case only boys) who have siblings have higher ND indicators, as well as the fact that the use of IF compared to breast milk is a statistically significant factor on the general ND of boys;
- Duration of breastfeeding does not affect ND;
- The number of family members affects children's ND.

IX. SCIENTIFIC PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS

IX.1. Publications:

1. Marinova M., Pancheva R., 2019, Nutritional prevention of allergies during pregnancy and breastfeeding, Medinfo 19, 52-56

2. Pancheva R, Marinova M., 2021, Some aspects of lactase deficiency in childhood. Medical News, 2021, 5, 7-9

3. Marinova-Achkar M, Toneva-Stoyanova A. Factors influencing the neurodevelopment of children with family history of allergy in North-Eastern Bulgaria. In Varna Medical Forum 2021 Dec 13 (Vol. 10, No. 2, pp. 181-186).

4. Nicolaou N, Pancheva R, Karaglani E, Sekkidou M, Marinova-Achkar M, Popova S, Tzaki M, Kapetanaki A, Iacovidou N, Boutsikou T, Iliodromiti Z. The Risk Reduction Effect of a Nutritional Intervention With a Partially Hydrolyzed Whey Formula-based cow's milk protein allergy and atopic dermatitis in high-risk infants during the first 6 months of life: the Allergy Reduction Study (ART), a multicenter, double-blind, randomized controlled trial. Limits in nutrition. 2022:985.

IX.2. Participation in scientific forums - international and national:

1. Marinova M. Feeding disorders in newborn with kernicterus - AHP ESPGHAN Summer School, Worcester College, Oxford, UK, 11-14.09.2019

2. Marinova M, N. Usheva, S. Eyubova, R. Pancheva. Factors influencing early childhood neuropsychological development in children from families at high risk for allergies. Fifth multifaceted conference on child development - Plovdiv, 02-04.04.2021

3. Marinova M, N. Usheva, S. Eyubova, R. Pancheva.. Atopic predisposition and developmental profile of children in early childhood in Bulgaria. 8th International conference on Nutrition and growth, 26-28.08.2021

4. Marinova M, N. Usheva, R. Pancheva, A. Toneva, S. Kuneva. Early childhood food allergy in families with atopy in Bulgaria, UEGW, 10.2021

5. Marinova M, R. Pancheva, N. Usheva, S. Eyubova. Neurodevelopment of children from atopic families in the early childhood in Bulgaria, 4-th International Pediamediterranea Conference, Istanbul, 11-13.05.2022

6. R. Chamova, M. Marinova, R. Pancheva, S. Popova, E. Karaglani, A. Toneva. Frequency of different forms of allergies among families at high risk for atopy in Varna. XV National Congress of the Bulgarian Pediatric Association – 23-26.09.2021

7. A. Toneva, M. Marinova, R. Pancheva, E. Karaglani, N. Usheva, L. Rangelova, R. Chamova. Influence of some risk factors related to the growth of children from atopic families during the first 1000 days. XV National Congress of the Bulgarian Pediatric Association - 23-26.09.2021

X. APPENDIXES

Appendix 1. Correlation analysis of the type of feeding during infancy and the anthropometric indicators of growth according to the age of the children (total) at 2, 4, 6 months and 2 years of age*

Type of milk feeding/duration	r/rho p	Weight at 2 m.	Length at 2 m.	Weight at 4 m.	Length at 4 m .	Weight at 6 m .	Length at 6 m.	TR 1 year	RV 1 year	TV 1 year	ITMV 1 year	TR 2 years	RV 2 years	TV 2 years	ITMV 2nd year
Exclusive breastfeeding - in months	r	0.10	-0.01	0.16	0.03	0.12	0.02	0.09	-0.06	0.06	0.09	-0.13	-0.05	-0.15	-0.12
	P	0.38	0.93	0.18	0.82	0.31	0.84	0.35	0.52	0.54	0.33	0.17	0.62	0.12	0.20
Breastfeeding -in total (months)	r	0.10	0.01	0.14	-0.01	0.09	0.03	0.07	-0.09	0.03	0.08	-0.14	-0.03	-0.14	-0.13
	p	0.35	0.94	0.23	0.93	0.46	0.83	0.46	0.35	0.76	0.41	0.16	0.76	0.14	0.18
Milk-kind	rho	0.03	0.13	-0.07	0.12	-0.003	0.08	-0.03	-0.02	-0.03	-0.02	**0.20**	0.00	0.18	**0.20**
	P	0.78	0.25	0.55	0.31	0.98	0.50	0.79	0.87	0.79	0.81	**0.03**	1.00	0.06	**0.035**
Breast-feeding-mixed-replacement	rho	-0.07	0.07	-0.04	0.11	-0.05	0.03	-0.02	-0.01	-0.02	-0.02	0.19	0.03	0.19	0.18
	r	0.53	0.52	0.75	0.37	0.69	0.83	0.83	0.95	0.85	0.85	0.05	0.77	0.05 1	0.056

* Correlation when controlling for smoking variables at mother's " birth " , number hospitalizations ” and “ total number of infections”

Appendix 2. Correlation analysis of the type of feeding during infancy and anthropometric indicators of growth of boys, adjusted for age - at 2, 4, 6 months and 2 years of age*

Type of milk feeding/ duration	**r/rho p**	**Weight at 2 m.**	**Height at 2 m.**	**Weight at 4 m.**	**Length at 4 m .**	**Weight at 6 m .**	**Height at 6 m.**	**TR 1 year**	**RV 1 year**	**TV 1 year**	**ITMV 1 year**	**TR 2 years**	**RV 2 years**	**TV 2 years**	**ITMV 2nd year**
Breastfeeding at all months	r	0.28	0.07	0.41	0.09	0.28	0.01	-0.08	-0.06	-0.11	-0.07	-0.01	-0.02	-0.02	-0.01
	p	0.06	0.64	**0.01**	0.57	0.09	0.97	0.52	0.63	0.40	0.57	0.95	0.86	0.86	0.94
Exclusive breastfeeding - in months	r	0.38	0.18	0.43	0.11	0.34	0.12	-0.09	-0.05	-0.11	-0.09	0.04	-0.06	0.00	0.04
	p	0.01	0.24	0.01	0.50	**0.04**	0.47	0.47	0.72	0.38	0.51	0.75	0.67	0.97	0.74
Milk-kind	rho	-0.21	-0.05	-0.39	-0.23	-0.30	-0.15	0.15	-0.02	0.15	0.14	0.08	-0.18	-0.08	0.13
	P	0.15	0.75	**0.01**	0.15	0.07	0.37	0.27	0.88	0.24	0.27	0.57	0.17	0.53	0.32
Breast-feeding-mixed-replacement	rho	-0.18	0.03	-0.24	-0.06	-0.24	-0.03	0.28	-0.11	0.22	0.29	0.11	-0.05	0.05	0.14
	r	0.25	0.83	0.14	0.71	0.15	0.87	**0.03**	0.41	0.09	**0.02**	0.41	0.68	0.72	0.28

**Correlation when controlling for the variables "mother's smoking", "birth", number of hospitalizations" and "total number of infections"*

Appendix 3. Correlation analysis of the type of feeding during infancy and the anthropometric indicators of growth according to age in girls at 2, 4, 6 months, 1- and 2-years of age *

Type of milk feeding/duration	**r/rho p**	**Weight at 2 months**	**Length at 2 m.**	**Weight at 4 m.**	**Length at 4 m .**	**Weight at 6 months**	**Length at 6 m.**	**WH at 1 year**	**HA 1 year**	**WA 1 year**	**IBMA 1 year**	**WH 2 years**	**HA 2 years**	**WA 2 years**	**IBMA 2 years**
Breastfeeding at all - months	r	0.23	0.17	0.12	0.20	0.11	0.20	0.28	-0.24	0.13	0.30	-0.03	-0.21	-0.18	-0.01
	P	0.15	0.28	0.49	0.25	0.55	0.27	**0.047**	0.09	0.37	**0.032**	0.81	0.13	0.21	0.93
Exclusive breastfeeding months	r	0.138	0.051	0.118	0.045	0.083	0.116	0.334	-0.248	0.174	0.357	-0.182	-0.131	-0.251	-0.167
	p	0.404	0.756	0.507	0.799	0.657	0.535	**0.018**	0.083	0.228	**0.011**	0.205	0.363	0.078	0.248
Milk-kind	rho	-0.213	-0.048	-0.393	-0.234	-0.297	-0.150	0.145	-0.020	0.154	0.144	0.075	-0.180	-0.082	0.129
	P	0.154	0.754	**0.012**	0.146	0.074	0.374	0.266	0.880	0.236	0.268	0.567	0.165	0.531	0.322
Breast-feeding-mixed-replacement	rho	-0.175	0.032	-0.236	-0.061	-0.243	-0.028	0.282	-0.107	0.218	0.290	0.108	-0.054	0.048	0.141
	r	0.246	0.831	0.143	0.707	0.147	0.868	**0.028**	0.413	0.091	**0.023**	0.408	0.678	0.716	0.279

****** Correlation between duration of breastfeeding and growth, controlled for ' Mother smoking yes / no ', ' Birth ', ' Number hospitalizations ', and 'Total number infections '*

Appendix 4. Comparative analysis of the total number of acute illnesses and their duration in boys, depending on exclusiveness and duration of breastfeeding in total

		NUMBER OF ACUTE INFECTIONS								DURATION (DAYS)								
		General number infections	Respiratory infections	Nasopharyngitis	Bronchiolitis	Laryngitis	Acute pneumonias	Acute gastroenteritis	Acute urinary infections	General duration on infections	Respiratory infections	Acute nasopharyngitis	Acute bronchiolitis	Acute laryngitis	Acute pneumonia	Acute gastroenteritis	Acute urinary infections duration-days	Other infections
Exclusive breastfeeding (months)	Pearson's r	-0.08	-0.08	-0.04	-0.09	0.21	-0.18	-0.10	0.16	-0.13	-0.15	-0.10	-0.09	0.20	-0.17	-0.09	0.25	-0.06
	p-value	0.55	0.57	0.79	0.51	0.10	0.17	0.43	0.23	0.32	0.27	0.47	0.49	0.12	0.19	0.49	0.06	0.63
Breastfeeding in total (months)	Pearson's r	-0.11	-0.10	-0.05	-0.11	0.21	-0.18	-0.12	0.13	-0.16	-0.18	-0.12	-0.12	0.19	-0.17	-0.11	0.23	-0.08
	p-value	0.40	0.45	0.68	0.40	0.10	0.18	0.37	0.32	0.21	0.18	0.35	0.35	0.15	0.18	0.41	0.08	0.54

Appendix 5. Comparative analysis of the total number and duration of acute illnesses and the number of hospitalizations of boys, depending on the way of feeding, kind of milk and type of feeding at birth

		NUMBER								**DURATION (DAYS)**									**HOSPITALIZATIONS**				
		General number infections	**Respiratory infections**	**Nasopharyngitis**	**Bronchiolitis**	**Laryngitis**	**Acute pneumonias**	**Acute gastroenteritis**	**Acute urinary infections _**	**General duration on the infections**	**Respiratory infections**	**Acute nasopharyngitis**	**Acute bronchiolitis**	**Acute laryngitis**	**Acute pneumonia**	**Acute gastroenteritis**	**Acute urinary infections**	**Others infections**	**Number hospitalizations**	**Respiratory _ infections**	**Gastroenteritis**	**Urinary infections**	**Others reasons**
Way of feeding breastfeeding/ substitute _	Spearman's rho	-0.15	-0.20	-0.26	0.03	-0.23	0.17	-0.06	0.13	-0.01	-0.03	-0.11	0.10	-0.22	0.17	-0.06	0.13	-0.06	0.03	0.03	-0.07	0.11	0.13
	p-value	0.26	0.13	**0.04**	0.81	0.08	0.19	0.67	0.31	0.94	0.80	0.39	0.47	0.09	0.20	0.63	0.31	0.68	0.83	0.84	0.58	0.41	0.31
Milk-kind	Spearman's rho	-0.09	-0.14	-0.14	0.07	-0.14	0.23	0.06	0.07	0.01	-0.01	-0.04	0.06	-0.18	0.23	0.07	0.07	-0.06	-0.05	-0.03	0.06	0.13	-0.07
	p-value	0.52	0.28	0.28	0.58	0.14	0.07	0.6 4	0.57	0.95	0.93	0.74	0.65	0.16	0.08	0.62	0.58	0.64	0.69	0.83	0.66	0.34	0.61
Feeding at birth	Spearman's rho	0.09	0.13	0.08	0.11	0.01	0.17	0.09	-0.01	0.18	0.17	0.19	0.17	0.01	0.17	0.10	-0.01	0.03	0.21	0.17	0.11	0.20	0.14
	p-value	0.48	0.31	0.54	0.41	0.95	0.20	0.48	0.9	0.18	0.19	0.15	0.20	0.92	0.20	0.44	0.93	0.82	0.11	0.19	0.40	0.12	0.31
Feeding at birth with/ without breast milk	Spearman's rho	-0.15	-0.20	-0.16	-0.10	-0.14	-0.10	-0.14	-0.09	-0.14	-0.13	-0.26	-0.14	-0.15	-0.07	-0.12	-0.09	-0.10	-0.18	-0.11	-0.13	-0.24	-0.09
	p-value	0.26	0.12	0.22	0.46	0.27	0.60	0.42	0.49	0.29	0.33	**0.048**	0.28	0.25	0.60	0.37	0.51	0.45	0.16	0.38	0.31	0.07	0.49

Appendix 6. Comparative analysis of the total number of acute illnesses and their duration in girls, depending on the exclusivity and duration of breastfeeding

		NUMBER								**DURATION (DAYS)**								**HOSPITALIZATIONS**			
		General number infections	**Respiratory infections**	**Nasopharyngitis**	**Bronchiolitis**	**Laryngitis**	**Acute pneumonias**	**Acute gastroenteritis-**	**Acute urinary infections**	**General duration of acute infections**	**Respiratory infections**	**Acute nasopharyngitis**	**Acute bronchiolitis**	**Acute laryngitis**	**Acute pneumonia**	**Acute gastroenteritis**	**Acute urinary infections**	**Number of hospitalizations**	**Hospitalizations for respiratory infections**	**Hospitalizations for gastroenteritis**	**Hospitalizations for urinary infections**
Exclusive _ breastfeeding (months)	Pearson's r	0.02	0.03	0.08	-0.08	0.03	0.02	0.01	0.26	-0.04	-0.03	0.04	-0.17	-0.02	-0.02	-0.06	0.02	-0.03	-0.17	0.11	0.35
	p-value	0.88	0.86	0.57	0.58	0.85	0.89	0.94	0.08	0.77	0.83	0.80	0.24	0.88	0.91	0.68	0.91	0.84	0.26	0.44	**0.014**
Breastfeeding – total duration – (months)	Pearson's r	-0.02	-0.04	0.06	-0.10	-0.02	-0.05	0.06	0.19	-0.14	-0.12	0.02	-0.24	-0.06	-0.12	-0.02	-0.03	-0.19	-0.31	0.03	0.30
	p-value	0.91	0.79	0.70	0.50	0.88	0.72	0.69	0.20	0.33	0.44	0.88	0.10	0.68	0.43	0.89	0.84	0.21	**0.033**	0.87	**0.037**

Appendix 7. Comparative analysis of the total number of acute illnesses and their duration in girls, depending on the way of feeding, kind of milk and type of feeding at birth

		Number								Duration (days)								Number of hospitalizations			
		General number infections	**Respiratory infections-number**	**Nasopharyngitis-**	**Bronchiolitis-**	**Laryngitis**	**Acute pneumonia**	**Acute gastroenteritis**	**Acute urinary infections**	**General duration of the infections-**	**Respiratory infections**	**Acute nasopharyngitis**	**Acute bronchiolitis**	**Acute laryngitis**	**Acute pneumonia**	**Acute gastroenteritis**	**Acute urinary infections**	**Number of hospitalizations**	**Hospitalizations for respiratory infections**	**Hospitalizations for gastroenteritis**	**Hospitalizations for urinary infections**
Way of feeding - breastfeedig /replacement	Spearman's rho	0.00	0.03	0.11	0.01	0.09	-0.19	-0.12	-0.02	-0.05	-0.01	0.07	0.15	0.05	-0.00	-0.11	-0.02	-0.12	0.08	-0.31	-0.17
	p-value	0.99	0.86	0.45	0.93	0.53	0.20	0.42	0.87	0.75	0.94	0.62	0.31	0.72	0.99	0.45	0.89	0.40	0.61	**0.03 0**	0.25
Milk-kind	Spearman's rho	0.00	0.08	0.06	0.09	0.19	-0.19	-0.0 7	-0.05	0.01	0.05	0.11	0.17	0.21	-0.09	-0.09	-0.04	-0.11	0.10	-0.31	-0.19
	p-value	0.99	0.56	0.66	0.50	0.19	0.17	0.61	0.72	0.94	0.71	0.45	0.24	0.15	0.55	0.53	0.76	0.46	0.49	**0.031**	0.18
Feeding at birth	Spearman's rho	0.02	0.01	-0.09	0.02	0.12	-0.11	0.00	-0.08	0.02	-0.03	-0.07	0.15	0.06	0.04	0.04	-0.07	0.01	0.20	-0.19	-0.19
	p-value	0.89	0.97	0.55	0.88	0.41	0.45	0.98	0.58	0.89	0.82	0.62	0.29	0.65	0.75	0.75	0.61	0.93	0.16	0.17	0.18
Feeding at birth with/ without breast milk	Spearman's rho	0.23	0.07	0.05	0.02	-0.02	0.21	0.18	0.20	0.12	0.13	-0.01	-0.10	0.05	0.03	0.12	0.20	-0.10	-0.22	0.21	0.13
	p-value	0.14	0.61	0.70	0.89	0.85	0.14	0.21	0.17	0.39	0.35	0.97	0.49	0.70	0.82	0.41	0.17	0.49	0.13	0.16	0.38

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