IMMUNOLOGICAL PROFILE OF THE CHILD AGED 1-23 MONTHS AND NATURALLY FED

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Abstract: *Child obesity is a public health problem. The onset may be early, even since the infancy. Recently, obesity has been considered an "inflammatory disease", but even the latest studies provide little data on the extent of the inflammatory process of adipose tissue in the child. Natural nutrition prevents obesity. Interleukin-6 and ultra-sensitive C-reactive protein are immunological markers associated with obesity. According to literature data, leptin directly influences the weight. The research aims the assessment of immune markers - leptin, interleukin-6 and C-reactive protein - in naturally-fed children, aged 1-23 months, including revealing the influence of immune markers on the weight of the subjects included in the study.*

INTRODUCTION

Child obesity has become a major public health issue - over 40 million children of school-age are obese, the observation being all the more serious as this increase is accompanied by that of diabetes mellitus type II, equally.(1)

Obesity is considered an "inflammatory disease" and for this reason, more and more studies are attempting to describe the immune response profile of obese children.(2,3)

Low-level inflammation is a feature of adult obesity, but even the latest studies offer little data on the extent of the inflammatory process of adipose tissue in the infant.

Studies provide evidence according to which there is no immunological milk composition produced by healthy women, living in different geographic, dietary and socio-economic areas. On the contrary, there are substantial inter- and intra-individual variations. However, the data suggests the existence of a common "base" set of immunoglobulins, cytokines, chemokines and growth factors that are present in mature milk produced by all women, regardless of their origin. Such physiological changes in the immunological profile of human milk may reflect individual patterns in the child's immune system.(4-8)

Ruiz and collaborators, in 2017, published the results of a comprehensive world study, focused on trying to develop an immune profile of human milk from healthy mothers. The concentrations of innate and acquired immunity factors, chemokines and growth factors in milk samples collected from healthy mothers from different geographic areas were analysed.(9)

Obesity predisposes to a pro-inflammatory condition through IL-6 and TNF-α raised inflammatory mediators and reduced levels of adiponectin. IL-6 is more related to the obese condition that influences the liver to synthesize and secrete C-reactive protein, which is a feature of systemic inflammation. Leptin is established as being directly related to weight.

The latest research in the field appreciates that:

- the six bioactive components of human milk are insulin, leptin, adiponectin, ghrelin, IL-6 and TNF-α;
- TNFα, IL-6 and ultra-sensitive C-reactive protein are the most studied inflammatory markers associated with obesity.(10-13)

Natural nutrition prevents obesity. The way in which natural nutrition "per se" prevents obesity is properly assessed by the entire immunological composition of the human milk. Unfortunately, there are independent, external factors that interfere with the beneficial role of natural nutrition in regulating body weight.

PURPOSE

The aim of the research is to evaluate interleukin-6, leptin and ultra-sensitive C-reactive protein as immune markers, in children aged 1-23 months old, naturally fed, included into the study.

MATERIALS AND METHODS

The prospective study, conducted in the Sibiu Paediatrics Clinic for a 24-month period, between January 2016 and December 2017, included 45 children aged between 1 and 23 months, naturally fed.

There were performed:

- anamnestic evaluation by collecting data related to family history, considering possible genetic, immunological, endocrinological diseases (mother's obesity) or chronic medication with indication for the mother; socio-economic conditions;
- anamnestic evaluation by collecting data related to the personal physiological antecedents (birth weight, parity, type of diet, correctness of diversification);
- anamnestic evaluation by collecting data related to the personal pathological history, taking into account possible genetic, immunological, endocrinological diseases (obesity other than nutritional) or chronic medication; data on a possible acute pathology in recent medical history (the last 7 days), requiring indication of anti-infective medication;
- clinical evaluation that allowed assessments of nutritional status (including weight and waist) and health status (signs and symptoms of an acute or chronic condition);
- biological evaluation that followed the determination/

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dosing of interleukin-6, leptin, ultra-sensitive C-reactive protein.

Exclusion criteria aimed at eliminating from the study the subjects with non-nutritional obesity, acute signs of infection or aggravation of chronic conditions that could influence the value of immune markers (especially interleukin-6 and ultra-sensitive C-reactive protein).

In order to determine weight percentiles, there were used the growth charts for 0-36-month-old child, for boys and girls, of the Centers for Disease Control and Prevention, 2000.

For dosing of ultra-sensitive C-reactive protein levels, the immunoturbidimetric method was used, the reference values being below 0.5 mg / dL.

The method used for **leptin** determination was an immunoenzymatic one, using the EIA technique.

Leptin reference values can be related to the body mass index (BMI), but also varies according to the laboratory-specified method of work (table no. 1).

Table no. 1. Leptin reference values according to BMI

BMI	Reference values in women (ng/mL)	Reference values in men (ng/mL)
18-25	<24	<10
26-27	6-32	1-25
28-29	8-50	2-23
30-31	11-68	3-36
32-33	14-90	5-56
34-35	19-121	8-70
36-37	25-141	12-135

The method used for the determination of interleukin-6 was immunochemical with electrochemiluminescence detection (ECLIA). The reference value is below 7 pg / mL.

RESULTS AND DISCUSSIONS

Depending on the weight percentiles, subjects included in the study were divided into two groups: group A - overweight or obese subjects (weight over percentile 90) and group B - subjects with normal weight (weight between percentiles 5 and 90).

Group A comprised 9 overweight or obese subjects and group B comprised 36 subjects with normal weight.

The results of inflammatory markers (leptin, interleukin-6 and ultra-sensitive C-reactive protein) are presented in table no. 2.

Table no. 2. Mean, median and mode of leptin, interleukin-6 and ultra-sensitive C-reactive protein values in subjects included in the study

Parameter	Mean	Median	Mode
Leptin(ng/mL)	1,41	0,6	0,4
Interleukin-6 (pg/mL)	15,94	2,69	1,5
Ultra-sensitive C-reactive protein (mg/dL)	0,36	0,211	0,012

It is found that at the level of the whole group, the mean of the leptin values is of 1.41 ng / mL, mean within the limits of normal. The median is of 0.6 ng / mL, the most common value being of 0.4 ng / mL.

The interleukin recorded a mean of 15.94 pg / mL, a value above the normal reference value. In contrast, the median is of 2.69 pg / mL, within the reference limits, and the mode is of 1.5 pg / mL.

The ultra-sensitive C-reactive protein was at a mean of 0.36 mg / dL, within the normal limits, the same as the median (0.211 mg / dL) and the mode (0.012 mg / dL).

Immune markers values were studied for each study group (see tables no. 2-3).

Table no. 2. Mean, median and mode of leptin, interleukin-6 and ultra-sensitive C-reactive protein values in subjects in group A

Parameter	Mean	Median	Mode
Leptin(ng/mL)	2,46	3,3	-
Interleukin-6 (pg/mL)	37,81	23,39	-
Ultra-sensitive C-reactive protein (mg/dL)	0,102	0,012	0,012

Table no. 3. Mean, median and mode of leptin, interleukin-6 and ultra-sensitive C-reactive protein values in subjects in group B

Parameter	Mean	Median	Mode
Leptin (ng/mL)	1,15	0,6	0,4
Interleukin-6 (pg/mL)	10,48	1,92	1,5
Ultra-sensitive C-reactive protein (mg/dL)	0,43	0,286	0,012

The analysis of the immune marker values in the study groups revealed the following:

- the mean of leptin values in group A is higher (2.46 ng / mL) than the mean of the values in group B (1.15 ng / mL), data in accordance with the specialty literature; the mean of values in group A is higher than the mean of the whole group (1.41 ng / mL);
- the mean of interleukin-6 values in group A is higher (37.81 pg / mL) than the mean of the values in group B (10.48 pg / mL), data in accordance with the specialty literature; the mean of values in group A is higher than the mean of the whole group (15,94 pg/mL);
- the mean of the values of the ultra-sensitive C-reactive protein in group A was lower (0.102 mg / dL) than the mean of the values in group B (0.43 mg / dL), inconsistent with literature data; the mean of values in group A is lower than the mean of the whole group (0,36 mg/dL).

It has been studied whether the differences in the value of immune markers are statistically significant (see table no. 4).

Table no. 4. Statistical significance of immune marker values in study groups

Parameter	Group A	Group B	p
Leptin(ng/mL)	2,46	1,15	0,02
Interleukin-6 (pg/mL)	37,81	10,48	0,29
Ultra-sensitive C-reactive protein (mg/dL)	0,43	0,286	0,24

It is found that only the value differences of leptin in groups A and B show statistical significance (p = 0.02), those of interleukin-6 and ultra-sensitive C-reactive protein not showing statistical significance. According to literature data, interleukin-6 values largely influence the values and tendency of ultra-sensitive C-reactive protein. Between the groups A and B the relationship has no statistical significance. (p = 0,39)

CONCLUSIONS

The study allowed the following conclusions:

- according to specialty literature data, leptin significantly influences the weight; children in group A (overweight or obese) showed significantly higher leptin values than children in group B (children with normal weight);
- neither the higher values of interleukin-6, nor those of ultra-sensitive C-reactive protein in group A (overweight or obese) compared to group B (children with normal weight) showed statistical significance; the explanation is provided by literature data according to which the existence of

subclinical infections or the incubation period of an acute infectious disease may interfere with the expected results and make it extremely difficult to recruit the subjects.

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