# Elevated soluble E-selectin concentrations in infants are associated with a short term of breast feeding, prematurity and intrauterine growth retardation

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***Abstract***

***Purpose:*** *Soluble adhesion molecules are the markers of early atherosclerotic changes. The aim of our study was to evaluate the concentrations of soluble adhesion molecules in blood sera of healthy children during first years of their life in relationship with a course of intrauterine life and the time of breast-feeding after birth.*

***Materials and methods:*** *The studies were carried out in 41 healthy children at age between 18 to 30 months. The concentrations of the soluble forms of intracellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) as well as soluble E-selectin were determined by a quantitative immunoenzymatic ELISA method.*

***Results:*** *The concentrations of soluble E-selectin were significantly higher in children breast fed for 3-6 months when compared to children who were breast-fed longer. The statistically significant negative correlations were confirmed between the concentrations of soluble E-selectin in sera of infants and a body weight and length at birth.*

***Conclusions:*** *The results of our investigations can suggest that elevated concentrations of soluble E-selectin in sera of infants can be associated with a short period of breast feeding, prematurity or intrauterine growth retardation*

***Key words:*** *sE-selectin, sVCAM, sICAM, infants*

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## Introduction

Atherosclerosis is a chronic disease of medium and big arteries and is characterized by complex lesions in the membrane of interior vascular wall [1, 2]. It should be emphasized that the initial period of atherosclerotic lesion may last for many years with no clinical manifestations on the onset. According to the contemporary opinions this condition may take place still during the early childhood [3, 4]. The atherosclerotic foci developing in the vessel wall result from the activity of many pathological processes. The most current reports indicate that the first stage of the atherosclerotic process is of inflammatory and immunological character. The local inflammatory reaction is a process that is closely related with the development and progress of atherosclerotic changes. The role of immunological response in the pathogenesis of atherosclerosis remains still unclear [5].

During the first phase of atherosclerotic process there are changes in the internal membrane of arteries, which are defined as fatty spots or fatty streaks. They are the earliest detected morphological atherosclerotic changes in the arteries. The fatty spot is composed of macrophages and

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lymphocytes T loaded with lipids. This process is beginning by the damage of vascular endothelium and this is manifested by its intensified permeability, expression of adhesive molecules on its surface and the decrease of production of nitric oxide. These processes on the area of internal membrane of the arteries result in collection of oxidized molecules of LDL lipoproteins. The presence of adhesive molecules makes monocytes and lymphocytes T adhesion to the endothelium possible and subsequently the migration into the vessel. Fatty streaks may appear in the vessels in early childhood and give rise to the atherosclerotic changes. This phase of atherosclerotic process is reversible. The atherosclerotic process starts when there are additional favouring situations, such as genetic conditioning, bioconstitutional conditioning and the influence of environment which may also modify genetic susceptibility for developing arteriosclerosis. [6].

Presently it is assumed that the dysfunction of the endothelial cells is one of the main pathogenetic factors of atherosclerosis [7-9]. Determining of adhesion molecules may be helpful in the diagnosis of early risk of cardiac ischaemia. The purpose of our study was to evaluate the concentrations of soluble adhesion molecules (intracellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin) in the blood serum of children during their first years of life depending on the course of intrauterine life and the time of breast-feeding after birth.

## Materials and methods

The studies were carried out in 41 healthy children at age between 18 to 30 months. On the basis of medical records and a questionnaire collected from the parents the gestational age, body mass and length were evaluated after birth. Also information were obtained on the method of child nourishment from the beginning of its life. The parents agreed in writing for exposing their children to the study. Biochemical tests in the serum were performed at he time of blood sampling when making the prescribed diagnostic or check-up tests in the outpatients clinics of Children University Hospital in Lublin. The blood for biochemical tests was sampled from the elbow vein on fast early in the morning. The blood was clotted at the room temperature and the serum was separated in laboratory centrifuge for 10-15 minutes with 2000-3000 rpm. The serum samples for determining soluble forms of adhesion molecules: soluble ICAM-1, soluble VCAM-1 and soluble E-selectin were stored at the temperature -20°C for 2-3 months.

The concentrations of the soluble forms of intracellular adhesion molecule-1 (sICAM-1) and vascular adhesion molecule-1 (sVCAM-1) as well as soluble E-selectin were determined by a quantitative immunoenzymatic ELISA method (Enzyme-linked immunosorbent assay). The assays were performed according to the procedure recommended by the manufacturer, with the application of kits: human sICAM-1 BMS201, human sVCAM-1 BMS232, human sE-selectin BMS205 (Bender Med Systems Diagnostics GmbH, Vienna, Austria).

The statistical analysis of the obtained results was done with application of Statistica 5.1 PL software (Stanisz 1998).

For the evaluation of the analysed properties and parameters in the study, the arithmetic mean (M), standard deviation (SD), mean error of arithmetic mean (SE) were used. All the variables were analysed for their distribution conformity with regular conformity with the application of Kołmogorow-Smirnow test, and subsequently the significance of differences between the mean values was evaluated.

When the characteristics had a regular distribution, the evaluation of statistical significance of the differences between studied groups was made with t-Student test. When there was discrepancy with the regular distribution, the non-parameter U Mann-Whitney test was applied.

The correlations between the studied factors and the selected indices were assessed with a correlation coefficient r-Pearson. The assumption of hypothesis in the statistical analyses was verified on the significance level $\alpha = 0.05$, so the differences for $p<0.05$ were considered statistically significant.

## Results

The mean concentrations of soluble VCAM-1 in male infants were higher than in female infants but the differences were not statistically significant (1798.97±661.53 ng/ml vs 1591.21±646.94 ng/ml; NS).

The concentrations of soluble ICAM were 354.51±75.88 ng/ml in male infants, they were 390.21±88.99 ng/ml in female infants but the differences were not significant either.

There were no differences between the concentrations of soluble E-selectin in male infants when compared to female infants (67.94±35.05 ng/ml vs 88.75±37.94 ng/ml.

The mean concentrations of sVCAM-1 were 1707.78±697.49 ng/ml in 18-month-old children; 1731.93±699.40 ng/ml in 24-month-old children and 1658.18±565.05 ng/ml in the 30-month-old children and the differences were not statistically significant.

The concentrations of soluble ICAM-1 were significantly lower in 18-month-old children when compared to 24-month-old children (344.37±10.0 vs 372.70±98.32 ng/ml; $p<0.05$). The highest values of soluble ICAM-1 concentrations were observed in 30-months-old children - 397.78±77.55 ng/ml.

The mean concentrations of soluble E-selectin were 87.67±43.48 ng/ml in 18-month-old children, 82.49±35.07 ng/ml in 24-month-old children. In 30-month-old children the mean concentrations were 60.11±27.63 ng/ml. They had the lowest values in this group of 30-month-old children

and the differences were statistically significant in comparison with 24-month-old children (p<0.01).

The concentrations of soluble VCAM were 1694.71±715.67 ng/ml in children breast-fed for the period of 3-6 months. In children who were breast-fed longer than 6 months they were 1716.53±559.66 ng/ml, but the differences were not statistically significant.

The concentrations of soluble ICAM in the breast-fed children until 3-6 month of life were lower (367.97±76.60 ng/ml) than in children who were breast-fed for a longer period (376.42±95.54 ng/ml) but these differences were outstandingly random.

The concentrations of soluble E-selectin were higher in children breast fed until 3-6 month of life when compared to children who were breast-fed for a longer time and the differences were statistically significant (82.25±42.11 ng/ml vs 69.50±27.25 ng/ml; p<0.05).

The statistically significant negative correlation was confirmed between the body birth weight and the concentration of soluble E-selectin in blood serum (r= - 0.375; p<0.05) and between body length at birth and the concentration of soluble E-selectin (r= - 0.320, p<0.05).

**Table 1.** The mean concentrations of soluble adhesion **molecules** in male and female infants

| Parameter (ng/ml) | gender | number | Mean ± SD | p |
|---|---|---|---|---|
| sVCAM - 1 | male | 22 | 1798.97±661.53 | NS |
| | female | 19 | 1591.21±646.94 | |
| s ICAM - 1 | male | 22 | 354.51±75.88 | NS |
| | female | 19 | 390.21±88.99 | |
| s E-selectin | male | 22 | 67.94±35.05 | NS |
| | female | 19 | 88.75±37.94 | |

**Table 2.** The mean concentrations of soluble adhesion molecules in three age groups of infants

| parameter (ng/ml) | infantile age (months) | number | mean ± SD | p |
|---|---|---|---|---|
| s VCAM - 1 | 18 | 13 | 1707.78 ± 697.49 | |
| | 24 | 16 | 1731.93 ± 699.4 | NS |
| | 30 | 12 | 1658.18 ± 565.05 | |
| s ICAM - 1 | [1] 18 | 13 | 344.37 ± 59.1 | p<0.05 |
| | [2] 24 | 16 | 372.70 ± 98.32 | 1:2 |
| | [3] 30 | 12 | 397.78 ± 77.55 | |
| s E-selectin | [1] 18 | 13 | 87.68 ± 43.48 | p<0.01 |
| | [2] 24 | 16 | 82.49 ± 35.07 | 1:3 |
| | [3] 30 | 12 | 60.11 ± 27.63 | |

**Table 3.** The mean concentrations of soluble adhesion molecules in relationship with the duration of breast feeding

| parameter (ng/ml) | duration of breast feeding (months) | number | mean ± SD | p |
|---|---|---|---|---|
| s VCAM - 1 | < 6 | 26 | 1694.71 ± 715.67 | NS |
| | > 6 | 15 | 1716.53 ± 559.66 | |
| s ICAM - 1 | < 6 | 26 | 367.97 ± 76.59 | NS |
| | > 6 | 15 | 376.42 ± 95.54 | |
| s E-selectin | < 6 | 26 | 82.25 ± 42.11 | p<0.05 |
| | > 6 | 15 | 69.50 ± 27.25 | |

**Table 4.** The correlations between the concentrations of soluble adhesion molecules and body weight and lenght at birth and gestational age

| Parameter (ng/ml) | | weight at birth | length at birth | gestational age |
|---|---|---|---|---|
| sVCAM - 1 | *r* | 0.037 | -0.072 | -0.020 |
| | p | p>0.05 | p>0.05 | p>0.05 |
| sICAM - 1 | *r* | 0.187 | 0.080 | 0.273 |
| | p | p>0.05 | p>0.05 | p>0.05 |
| sE-selektyna | *r* | -0.375 | -0.320 | -0.265 |
| | p | **p<0.05** | **p<0.05** | p>0.05 |

The concentrations of selected adhesion molecules in the blood of male and female infants are presented in Table 1. Table 2 presents the concentrations of endothelial adhesion molecules in the three age groups of children. The concentrations of adhesion molecules in the blood serum of children depending on the period of breastfeeding are presented in Table 3.

The correlation coefficients between birth weight and body length, gestational age and the selected adhesion molecules – markers of early atherosclerotic changes in children during their first years of life are presented in Table 4.

## Discussion

It has been suggested lately that the pathogenesis of atherosclerosis is associated with the chronic inflammation of endothelial cells. This process can lead to a progression of early atherosclerotic lesions. There is an agreement that the activation or the dysfunction of endothelium is one of the pathogenic factors of atherosclerosis. The dysfunction of endothelial cells leads to the initiation and progression of vascular diseases and causes their clinical signs [10]. The activated endothelial cells produce soluble adhesion molecules, such as: soluble ICAM-1, soluble VCAM-1 and soluble selectin-E [11]. The results of clinical studies can confirm the role of these molecules in the pathogenesis of atherosclerosis.

In our study we estimated the concentrations of soluble E-selectin, soluble ICAM-1 and soluble VCAM-1 in sera of infants to detect early atherosclerotic alterations. We also estimated the correlations between the concentrations of soluble adhesion molecules and body weight and lenght at birth and gestational age.

The mean concentrations of soluble adhesion molecules in sera of children at the age between 18 to 30 months were following - sVCAM-1 1702.67±663.01 ng/ml, soluble ICAM-1 371.06±84.12 ng/ml, soluble E-selectin 77.58±77.58±37.88 ng/ml and there were no significant differences in the relationship with a gender of infants. The concentrations of soluble VCAM-1 were similar in the children at the age of 18, 24 and 30 months. The concentrations of soluble ICAM-1 were increasing in the consecutive age groups and they have reached the highest values in the group of 30-month old children. The highest concentrations of soluble E-selectin have been observed in the group of 18-month old children and the lowest concentrations in in the group of 30-months old children.

The analysis of the correlations between soluble adhesion molecule concentrations and the duration of the brest feeding revealed that there were higher concentrations of soluble E-selectin in the group of children who were brest-fed for the period of 3-6 months. There were no correlations between the concentrations of soluble VCAM-1 and ICAM-1 and the duration of the breast feeding.

Furthermore, we observed the negative correlation between the concentrations of soluble E-selectin and body weight and lenght at birth. There were no correlations between the concentrations of sVCAM-1 and sICAM-1 and body weight and length at birth.

In the long-term study conducted by Nash et al. the concentrations of soluble forms of E-selectin, ICAM-1, VCAM-1 were estimated in the children between the age 9.5 to 15.5 [12]. They observed that the concentrations of soluble adhesion molecules were decreasing along with the age of the children. The similar investigations in the group of healthy infants revealed that the concentrations of soluble adhesion molecules did not change during first months of their life [13]. The alterations in the concentrations of soluble adhesion molecules have been observed in the group of neonates with the inflammatory processes or with the disturbances in the haemostasis system [13].

The concentrations of soluble E-selectin in the sera of healthy, full-term neonates estimated in the second and fifth day of the neonatal life were higher in the newborns when compared to the concentrations of soluble E-selectin in adults. There were no correlations between the concentrations of soluble E-selectin and gender, neonatal weight at birth, a kind of a delivery and the age of mothers [14]. The investigations carried out by Giannaki et al. revealed that the concentrations of soluble ICAM-1 were elevated in healthy neonates between first and thirty day of their life [15].

Furthermore, it has been observed that the concentrations of serum soluble intracellular adhesion molecule-1 can by affected by a time of day, food intake, physical and psychological stress [16].

The concentrations of soluble ICAM-1 are elevated in children during the HIV infections, hepatitis C, bronchial asthma, keratoconjunctivitis and pediatric connective tissue diseases [17-21]. However, the concentrations of soluble VCAM-1 are elevated during cytomegalovirus infection and during the sickle cell acute chest syndrome [22, 23]. The concentrations of soluble ICAM-1, soluble VCAM-1 and soluble E-selectin are increased in the plasma of children with sepsis-induced multiple organ failure [24]. The authors propose the evaluation of these molecules for the monitoring of treatment in children with Staphylococcus aureus bactaeriemia or sepsis [24, 25]. It has been shown lately that atherosclerotic process can start in early childhood. The progression of the atherosclerotic process depends on many risk factors of ischaemic heart disease. The adhesion molecules play an important role during the initial phases of atherosclerotic process. Moreover, the expressions of the adhesion molecules can be higher in children when compared to adults. The correlations between soluble adhesion molecule concentrations and parameters of lipid metabolism suggest that there is a need to eliminate risk factors of coronary artery disease in children [26]. The protection against coronary artery disease should start very early during a childhood to prevent elevated expressions of adhesion molecules in endothelial cells.

We observed higher concentrations of soluble E-selectin in infants who were brest fed during a period shorter than 6 months and in infants who had a low body weight and length at birth. The results of our investigations can suggest that elevated concentrations of soluble E-selectin in sera of infants can be associated with a short period of breast feeding, prematurity or intrauterine growth retardation.

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