**Introduction**

Leukocyte adhesion is a crucial step in the development of both normal immune response and inflammation (4,9). Adhesion of leukocytes is mediated through the multiple interactions between adhesion molecules and their ligands. Recently we have learned that soluble isoforms of these adhesion structures can be found in the circulation and their levels can serve as markers of disease activity (7,21). The information obtained from the measurement of soluble adhesion molecules can be interpreted in several different ways. The expression and subsequent release of soluble cellular adhesion molecules is mediated by proinflammatory stimuli both exogenous and endogenous origins such as endotoxin, histamin, thrombin, and various cytokines. Soluble cellular adhesion molecules may be regarded simply as the markers of the presence and intensity of inflammation. The clinical utility of monitoring levels of soluble adhesion molecules is not yet established, but it is suggested that the availability of commercial assay kits should allow their evaluation in many clinical settings (8). There is a demand for studies that would delineate the parameters of the immune system in healthy population and establish their normal ranges for clinical use. Particularly interesting is the situation in children’s population where age trends may be expected and blood samples are not readily available. The aim of this study is to establish normal ranges for serum levels of sICAM-1, sE-selectin and sVCAM-1 and their age related changes in healthy children’s population from 6 to 15 years of age.

**Materials and Methods**

**Subjects**

The serum samples were obtained from 158 healthy children (81 females, 77 males) aged from 6 to 15 years. The median age was 10 years. There were at least ten children in any one-year interval examined. Serum samples were drawn for the purpose of the vaccination surveillance study organized by Military Medical Academy in Hradec Králové, Czech Republic. Neither additional venipuncture nor larger volume of blood was required. The adult group consisted of 70 healthy blood donors (25 females and 45 males, median age 46 years). All subjects were interviewed and examined by the physician before blood sampling. This study was approved by the institutional ethical committee and informed consent was obtained from parents of all participants in this study.
**Blood sampling**

Peripheral blood samples were collected by the venipuncture into sterile tube (Sarstedt, Germany). Blood samples were collected between 8 and 9 hours a.m. after 10 hours of fasting and after 15 min of rest. All participants were asked in advance to avoid unusual physical load for 12 hours before blood drawing. Blood was left at room temperature for 1.5 hours. Serum samples were collected after centrifugation and were stored frozen at -40 °C up to 3 months and thawed only once immediately before processing.

**Measurement of soluble forms of adhesion molecules**

Serum concentrations of sICAM-1, sVCAM-1 and sE-selectin were obtained by use commercial ELISA kits Parameter Human Soluble ICAM-1, Parameter Human Soluble VCAM-1 and Parameter Human Soluble E-selectin manufactured by RD Systems (Minneapolis, MN, USA). ELISA technique was performed according to the manufacturers’ instructions. Briefly, diluted samples, standards and controls were incubated in microtiter wells coated with monoclonal antibodies anti-human sE-selectin, sICAM-1 or sVCAM-1. Coupled human sICAM-1, sE-selectin and sVCAM-1 were then detected by secondary antibody conjugated to horseradish peroxidase. Final color reactions were measured by Multiscan MCC 340 reader (Flow Lab, UK) and data were processed automatically. All measurement were done with the same batch and in a duplicate.

**Statistical analysis**

The analysis was done using SigmaStat 2.0 statistical software (Jandel Corporation, USA). The normal distribution of data was proved. The differences between groups were calculated by t-test or Mann-Whitney Rank Sum test and correlation analysis was performed according to Pearson or Spearman tests.

**Results**

The statistical differences between genders in the whole group were tested by the nonparametric Mann-Whitney Rank Sum Test. No statistical differences in any parameter tested (sICAM-1, sVCAM-1 and sE-selectin) were found. The possibility that these parameters are age-related was evaluated. The statistically significant decline in the level of sICAM-1 (coefficient of correlation r = -0.326, p < 0.001) and E-selectin (coefficient of correlation r = -0.283, p < 0.001) was found in contrast to the level of sVCAM-1 which remained unchanged (r = -0.056, p < 0.05). Similar statistically significant age-related correlations for both sICAM-1 (males r = -0.341, p=0.002, females r = -0.296, p=0.007) and sE-selectin (males r=-0.241, p=0.035, females r=-0.331, p=0.003) were reached when the group was subdivided according to the gender. No such correlation was found for sVCAM-1. Age-related trends in the levels of soluble adhesion molecules are shown in Fig. 1-3.

The optimal subdivision of the whole examined group to the intervals of age according to the trends in the levels of soluble adhesion molecules sICAM-1 and sE-selectin was calculated. The testing was started at the age of six years and was increased gradually within the interval of one year. The correlation with age was always calculated. No statistical correlation of age and the levels neither sICAM-1 nor sE-selectin was found for the 6 - 10 years old children. This fact was the reason to establish the first reference group. The second group was delineated by the same approach for children 11 - 15 years old. The calculated relations were confirmed by additional statistical tests. Statistically significant differences in the serum level of both sICAM-1 (p < 0.001), and sE-selectin (p=0.005) but not for the level of sVCAM-1 (p=0.320) were calculated.

![Fig. 1](image1.png) **Fig. 1:** Relationship between serum levels of sICAM-1 and age in the whole group of children (6-15 years old). The solid line represent regression curve.

![Fig. 2](image2.png) **Fig. 2:** Relationship between serum levels of sE-selectin and age in the whole group of children (6-15 years old). The solid line represent regression curve.
belonging into the families of immunoglobulins and integrins through the interaction between adhesion molecules could be bound through this interaction. Next step is medi-Granulocytes, monocytes and a subset of memory T cells of leukocytes e.g. CD15 molecule of granulocytes. E-selectins on the surface of activated endothelial cells and of inflammation. It is mediated by the interactions between rolling along the activated endothelial cell lining of postcapillary venules are the earliest signs of inflammation. Rolling is an essential element of the multistep cascade leading to the leukocyte recruitment into sites of inflammation. It is mediated by the interactions between E-selectins on the surface of activated endothelial cells and their ligands, which are heavily glycosylated surface molecules of leukocytes e.g. CD15 molecule of granulocytes. Granulocytes, monocytes and a subset of memory T cells could be bound through this interaction. Next step is mediated through the interaction between adhesion molecules belonging into the families of immunoglobulins and integ-
school age children (13). In another study, values of these three soluble cell adhesion molecules in 48 healthy children (median age 5 years) were found to be approximately double of those seen in adults (16). Similar results were obtained in study of Sack and co-workers (20). Nash and co-workers in a large study observed significant correlation of levels of sE-selectin, sICAM-1 and sVCAM-1 with age between 9 and 16 years at regular 2-year intervals (17). These results are very similar to our own findings concerning sICAM-1 and sE-selectin. In case of sVCAM-1 our result are not consistent with this study. There was no significant correlation of sVCAM-1 and age in children from 6 to 15 years, despite the fact that the levels of sVCAM-1 were significantly higher in 10 - 15 years old children compared to adults (p<0.001).

The physiological role of changes in the levels of soluble adhesion molecules during maturation is so far unknown. Great care must be taken in analysis and interpretation of obtained data, particularly if the obtained distribution is not normal. It has been suggested that 200 adult samples should be used to determine reference range. The numbers of samples that would be needed to establish reference values for children in narrowly defined age groups are not feasible. However, to circumvent this problem, we provided a 95% confidence interval for both the lower and upper limits of ranges obtained. While it does not increase the precision of data in a manner that an increase in sample size would, the confidence interval does give a good indication of the accuracy of the presented values and makes the data useful for comparison purposes. Appropriate age-dependent control groups are essential in any study of these molecules in children. Ideally, each laboratory should establish their own reference ranges. The appropriate age related values must be known for the markers of interest to avoid the misinterpretation of obtained data and finally the faulty clinical decision.

References


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