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Outpatient Consulting Unit for Children of Diabetic Mothers
Children's University Hospital in Lublin
Department of Pulmonary Diseases and Rheumatology
Department of Clinical Immunology, Medical University of Lublin

JACEK POSTĘPSKI, JACEK TABARKIEWICZ, AGNIESZKA MAJCHER, VIOLETTA OPOKA-WINIARSKA, EWA TUSZKIEWICZ-MISZTAL, JACEK ROLIŃSKI

The evaluation of the adhesive molecules' levels in diabetic mothers' children

Type I diabetes belongs to autoimmune diseases, which is characterised by disturbances in the cellular response. In genetically predisposed patients, as a result of the inflammatory infiltration from the immune-competent cells, there appears an immune-dependent destruction of islet β-cells, an organ specific humoral reaction appears, too. There emerge specific antibodies against such auto-antigens as glutamic acid decarboxylase (anti- GAD), protein tyrosine phosphatases (IA-2A) or insulin (IAA), and also against unidentified islet â cells antigens (ICA) or neuro-ectodermal cells antigens (1, 9).

The process of mononuclear cell migration from the vascular lumen to the inflammation site is mediated by specific receptor proteins of endothelium, the lymphocytes and the cells presenting the antigen – the so-called adhesive molecules. They are non-specific indicators of inflammation. Their presence in the majority of cells of the inflammatory infiltration of islet β-cells testifies undoubtedly to their participation in the reaction of the leukocytic system with islet β-cells, though their role does not seem clear. Simultaneously, apart from the particles present in the blood serum cells, their soluble analogues appear which, most probably, are freed to the circulatory system through the activated endothelial cells, lymphocytes and blood platelets (3, 10).

In patients with recently diagnosed diabetes and in their Ist degree relatives, an elevated level of these molecules can be found. The studies conducted so far have pointed to the possibility of taking into consideration the presence of soluble adhesive molecule forms such as sP- selectin, sICAM-1 cell adhesion molecule and sVCAM-1 vascular adhesion molecule as some of the possible indicators of prediabetis stage in Ist degree relatives, who constitute the risk group of type I diabetes (15).

Selectins belong to the group of adhesive proteins with lectin in their molecule and are divided into three basic groups: sL-selectins, sP-selectins and sE selectins, depending on the cell type they occur in. P-selectin is present in the membranes of blood platelets and on Weibel-Palade's bodies of endothelial cells; it participates in adhesion of poly-nuclear neutrophiles to the endothelium and participates in the processes along with pro-inflammatory cytokines; it activates integrine expression on leukocytes. sL-selectin is present in all leukocytes and conditions their adhesion to specific ligands on activated endothelial cells of the peripheral lymphatic nodes. It is responsible for leukocytes rolling along the endothelial cells. sE-selectin is present in the endothelial cells and its activity depends on stimulation through pro-inflammatory interleukines. (7, 10). sICAM-1 intercellular adhesion molecule

belongs to the super-family of immunoglobulins and in combination with the specific ligand – LFA-1 integrin in leukocyte membrane, causes it adhesion to the inflammation-affected endothelium (1, 10). sICAM-3 intercellular adhesion molecule is also an immunoglobulin membrane receptor with great similarity to sICAM-1 intercellular adhesion molecule in its external structure. sLFA-1 also has its ligand. It is present in lymphocytes, monocytes and neutrophiles but in distinction to ICA-1 does not prevail in endothelial cells. sICAM-3 is considered to play a role in immune response. sVCAM-1 vascular adhesion molecule also belongs to the super-family of immunoglobulins and similarly to sICAM-1 in combination with its ligand- sVLA-4 integrin mediates in adhesion of the leukocyte to the endothelium, mediated by inflammatory cytokines (I1-1, I1-4, I1-13, TNFα, INF-γ). (1, 5, 10).

The aim of the study was investigation of the consecutive indicator of developing diabetes in *prediabetes* period. For this reason the children of type 1 diabetic mothers were screened for blood serum levels of soluble adhesive molecules.

MATERIAL AND METHODS

The study comprised a group of 68 children of diabetic mothers (Type 1 DM) all of them – the patients of the Outpatient Consulting Unit for Children of Diabetic Mothers at Children's University Hospital in Lublin (Poland). The group consisted of 36 boys and 32 girls, aged from 1.5 to 16.5 years average of age 5.96 ± 3.8 years. The 33.3% of the investigated group of children were born prematurely. In 5 children (8.5%) typical traits of birth macrosomia were found. At examination obesity was identified in 2 children (BMI >25kg/m^2) and one child was overweight (BMI in the range of 22–25kg/m^2). On the day of blood samples collecting, children underwent subjective medical examination – none of them presenting any symptoms of infection. The results were compared with those obtained from control group of 20 healthy children without any history of type 1 diabetes in the medical interview.

The study was carried out according to DS.261/04 research project of Medical University of Lublin following the permission of Bioethical Commission of the University.

In the investigated groups the levels of sL-selectin, sP-selectin, sE-selectin, sVCAM-1, sICAM-1 and sICAM-3 were assessed. The evaluation of the researched molecules was performed at the Department of Clinical Immunology of Medical University of Lublin. The levels of the investigated adhesive molecules were determined by the use of immunoenzymatic assays (ELISA) f. *Bender MedSystems.*

Statistical analysis. The normality of level distribution of the soluble forms of adhesive molecules was checked by the use of W Shapiro-Wilk test. Regarding the fact that the distribution of the investigated features was different from the normal distribution, the levels of antibodies were expressed as median values and the direction and force of asymmetry of the feature distribution was described as the slant coefficient. For the comparison of the levels of the examined antibodies in specific groups, non-parametric Mann-Whitney test was used. Statistical significant value was described as $p< 0.05$.

RESULTS

The results are presented in Table 1. The levels of soluble forms of sL-selectin, sE-selectin, and sVCAM-1, sICAM-1, and sICAM-3 in diabetic mothers' (type 1) children and in healthy children did not differ statistically ($p< 0.05$). In studied diabetic mothers' (type 1) children the level of soluble sP-selectin was lower than in control group and the difference was significant. ($p> 0.05$).

Table 1. Serum level sL-selectin, sP-selectin, sE-selectin, and sVCAM-1, sICAM-1, and sICAM-3 in diabetic mothers (type 1) children and the healthy controls (presented as median values)

Adhesion molecules	Units	Controls n=20	Children of diabetic (T 1) mothers n=68	p
sL-selectin	ng/ml	2137.4	2312.4	p>0.05
sE-selectin	ng/ml	79.2	62.7	p>0.05
sP-selectin	ng/ml	226.4	115.1	p<0.05
sICAM - 1	ng/ml	706.6	703.7	p>0.05
sICAM - 3	ng/ml	51.4	51.1	p>0.05
sVCAM -1	ng/ml	1297.8	1425.8	p>0.05

DISCUSSION

Despite significant differences in opinions on ethio-pathogenesis of type I diabetes all current concepts assume genetically pre-conditioned proneness to islet β-cells cell impairing molecules and point to the role of the environmental factors. The result of these factors action is activation of the immune-competent cells, disturbances in cytokine balance, the change of cell adhesion molecules expression. The destruction process of islet β-cells starts, which is reflected in the appearance of antibodies against the antigens of these cells. The fundamental indicator of this autoimmune reaction is the appearance of antibodies directed against glutamic acid decarboxylase (anti GAD) and also against other pancreatic enzymes: tyrosine phosphatase (IA-2) and unidentified islet antigens (ICA) as well as insulin antibodies (IAA). The initiated auto-destructive process results in *prediabetes* stage in which all the above described disturbances of the immune system as well as disturbances in glucose tolerance take place. Finally, immunological auto-destructive process ends with the compensation inability of the organism and type I diabetes clinical symptoms. The fact of existence of a genetic predisposition for type I diabetes puts forward a group of the closest relatives (I, II degree) as the risk group. The frequency of type I diabetes occurrence in the offspring of diabetic mothers is estimated as 2–3% (13).

The unique, commonly accepted way of evaluation of the immunological reaction and describing the risk level of all-symptom type I diabetes is the evaluation of the panel of basic antibodies directed against islet β-cells antigens supplemented by evaluation of insulin secretion of the first phase in the test with *IV* administered glucose. The evaluation of cellular adhesion may be an additional marker of the starting *prediabetes* (6, 14). Such conclusions may be drawn from the research by Myśliwiec et al. (11), who found the elevated ICAM levels both in patients with recently diagnosed diabetes as well as in individuals with *prediabetes*. A similar role of sP-selectin is stressed by Baraniak et al. (1).

In our study we tried to formulate the question whether in type I diabetic mothers' children studied by us, in the asymptomatic period of diabetes, at the correct tolerance of carbohydrates (testified with normal values of oral test glucose loading and with the correct glycosil haemoglobin values) one is able to diagnose immunological symptoms of starting *prediabetes*. The levels of the soluble forms of the studied adhesive molecules were not much different from the levels obtained in the control group. Despite the fact that in 68 children studied in 12 the presence of at least 1 of GADA and/or IA-2A antibodies was confirmed, no correlation between their occurrence and a possible disturbance of adhesive molecules levels or disturbances in carbohydrate tolerance should be mentioned (12). Our studies did not confirm elevated levels of adhesive molecules in blood serum observed by other researchers. Manderson J.G. et al. (8) report an elevated level of ICAM and sE-selectin in examined 57 children of diabetic mothers. Similar results were achieved by Baraniak A. et al. (1). They all point

to the elevated ICAM-1 and sP-selectin expression both in 1st degree diabetic relatives and in patients with clinically developed diabetes. The patients' age in both cited studies was much higher and in a study by Baraniak et al. (1), among the studied subjects only L constituted diabetic mothers' children. It is acknowledged that a high level of adhesive molecules is observed in patients with recently diagnosed diabetes (4, 6). These observations were concerned with sP-selectin and sL-selectin as well as intercellular and vascular adhesion molecules. In the course of the illness, however, normalization of these values could take place (1).

The role of adhesive molecules stressed by Manderson J.G et al (8) as the factor responsible for arteriosclerosis development is worth noticing. Such a broad view on determination of adhesive molecules levels in diabetic mothers' children provides a new insight into the problem. Introducing the principles of elementary prevention in these patients may not only delay diabetes but also lower the risk of arteriosclerosis and circulatory illnesses. Although in our study group the levels of most estimated adhesive molecules of children of diabetic mothers (Type 1 DM) were not different from those obtained from the healthy children the follow up of this group in the future appears to be most necessary; carrying out further research also appears a must.

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SUMMARY

Type 1 (insulin-dependent) diabetes mellitus is a chronic autoimmune disease in which the immunnological tolerance to pancreatic islets ß-cells' autoantigens is damaged. Due to environmental factors an activation of immune-competent cells, cytokine balance disturbance, a change in the expression of cells adhesion molecules and appearance in serum antibodies to islets b-cells' antigens all take place. It is firstly reflected in the *prediabetes* stage of illness and finally in a type 1 diabetes mellitus (Type 1 DM) clinical symptoms occurrence. The aim of the study was to estimate the soluble forms of sL-selectin, sP-selectin, sE-selectin, sVCAM-1, sICAM-1 and sICAM-3 adhesive molecules in blood serum, as the early markers of *prediabetes* stage of illness, in population of children of diabetic mothers (Type 1 DM). The group of 68 children of diabetic mothers (Type 1 DM)was investigated. The group comprised 36 boys and 32 girls aged 1.5–16.5 (mean 5.96±3.8) years. The levels of the soluble forms of the adhesive molecules were measured using immunoenzymatic assays (ELISA) f. *Bender MedSystems*. The results were compared with those obtained from control group of 20 healthy children. The levels of: sL-selectin, sP-selectin, sE-selectin, sVCAM-1, sICAM-1 and sICAM-3 in the studied children of diabetic mothers (Type 1 DM) and in control group were similar and the differences were not significant ($p>0.05$). The level of the sP-selectine was lower than the one obtained from control group. The difference was significant ($p<0.05$). Although in our study group the levels of most estimated adhesive molecules of children of diabetic mothers (Type 1 DM) were not different from those obtained from the healthy children the follow up of these group in the future is still necessary regarding their genetic predisposition to the illness.

Ocena stężeń cząsteczek adhezyjnych u dzieci matek chorych na cukrzycę

Cukrzyca typu 1 jest przewlekłą chorobą autoimmunologiczną, w której dochodzi do destrukcji komórek β wysp trzustkowych. W wyniku działania czynników środowiskowych dochodzi do uaktywnienia komórek immunokompetentnych, zaburzenia równowagi cytokinowej, zmiany ekspresji cząsteczek przylegania na komórkach oraz pojawienia się w surowicy przeciwciał przeciw antygenom komórek β wysp trzustki. Zapoczątkowany proces autodestrukcyjny doprowadza do wystąpienia okresu *prediabetes*, a następnie do pełnoobjawowej cukrzycy typu 1. Celem pracy było poszukiwanie u dzieci matek cukrzycowych wykładników rozwijającej się cukrzycy w fazie przedklinicznej, jakimi mogłaby być obecność w surowicy krwi rozpuszczalnych form cząstek adhezyjnych: sL-selectin, sP-selectin, sE--selectin, sVCAM-1, sICAM-1 i sICAM-3. Badaniem objęto 68 dzieci matek chorych na cukrzycę typu 1. W badanej grupie było 36 chłopców i 32 dziewcząt w wieku 1,5–16,5 (średnio 5,96±3,8) lat. Stężenia badanych rozpuszczalnych form cząstek adhezyjnych były oznaczane metodą immunoenzymatyczną (ELISA) przy użyciu zestawów f. *Bender MedSystems*. Wyniki odnoszono do uzyskanych w grupie kontrolnej 20 zdrowych dzieci. Stężenia sL-selectin, E-selectin, sVCAM-1, sICAM-1 i sICAM-3 nie różniły się od uzyskanych w grupie kontrolnej ($p>0,05$). Stwierdzono jedynie istotnie niższy poziom sP--selektyny ($p<0,05$). Jakkolwiek u badanych przez nas dzieci matek cukrzycowych stężenia większości badanych cząstek adhezyjnych nie różniły się od stężeń uzyskanych w grupie kontrolnej, to jednak uwzględniając ich obciążenie genetyczne, dzieci te wymagają dalszej obserwacji.