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# The Study of Glycative and Oxidative Stress in Type 1 Diabetes Patients in Relation to Circulating TGF-Beta1, VCAM-1 and Diabetic Vascular Complications

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## 1. Introduction

Type 1 diabetes mellitus (T1DM) is one of most frequent autoimmune diseases and is characterized by absolute or nothing short of absolute endogenous insulin deficiency which results in hyperglycemia that is considered to be a primary cause of diabetic complications (DC) (Rambhade et al., 2010). T1DM leads to various chronic micro- and macrovascular complications. Diabetic nephropathy is a major cause of morbidity and mortality in patients with DM. Microvascular disease is the main determinant in the development of late complications in DM.

Persistent hyperglycemia is linked with glycation, glycooxidation, and oxidative stress (Aronson, 2008; Negre-Salvayre et al., 2009). During glycation and glycooxidation there are formed early, intermediate and advanced glycation products via Maillard reaction, glucose autooxidation and protein glycation. Accumulation of advanced glycation end products (AGEs) has several toxic effects and takes part in the development of DC, such as nephropathy (Kashihara et al, 2010), neuropathy, retinopathy and angiopathy (Peppas & Vlassara, 2005; Yamagishi et al., 2008; Goh & Cooper, 2008; Karasu, 2010). Higher plasma levels of AGEs are associated also with incident cardiovascular disease and all-cause mortality in T1DM (Nin et al., 2011). AGEs are believed to induce cellular oxidative stress through the interaction with specific cellular receptors (Ramasamy et al., 2005; Boulanger et al., 2006; Yamagishi, 2009; Mosquera, 2010). On the other side, carbonyl stress-induced tissue damage is caused by AGE precursors formed by hyperglycaemia, hyperlipidemia, nonenzymatic glycation, peroxidation of lipids and metabolic processes.

It has been suggested that the chronic hyperglycaemia in diabetes enhances the production of reactive oxygen species (ROS) from glucose autoxidation, protein glycation and glycooxidation, which leads to tissue damage (Son, 2007). Also, cumulative episodes of acute hyperglycaemia can be source of acute oxidative stress. A number of studies have summarized the relation between glycation and oxidation (Boyzel et al., 2010). The overproduction of ROS leads to oxidative modification of biologically important compounds and damage of them. Uncontrolled production of ROS often leads to damage of cellular macromolecules (DNA, lipids and proteins).

Some oxidation products or lipid peroxidation products may bind to proteins and amplify glycooxidation-generated lesions. Lipid peroxidation of polyunsaturated fatty acids, one of the radical reaction *in vivo*, can adequately reflect increased oxidative stress in diabetes. Advanced oxidation protein products (AOPP) are formed during oxidative stress by the action of chlorinated oxidants, mainly hypochlorous acid and chloramines. In diabetes the formation of AOPP is induced by intensified glycooxidation processes, oxidant-antioxidant imbalance, and coexisting inflammation (Piwowar, 2010a, 2010b). AOPP are supposed to be structurally similar to AGEs and to exert similar biological activities as AGEs, i.e. induction of proinflammatory cytokines in neutrophils, as well as in monocytes, and adhesive molecules (Yan et al., 2008). Accumulation of AOPP has been found in patients with chronic kidney disease (Bargnoux, et al., 2009). Further possible sources of oxidative stress are decreased antioxidant defenses, or alterations in enzymatic pathways.

Diabetes is associated also with inflammation (Navaro & Mora, 2006; Wautier et al., 2006; Devaraj et al., 2007; Hartge et al., 2007; Fawaz, et al., 2009 ; Van Sickle et al., 2009; Nobécourt et al., 2010). ROS are implicated also in the pathogenesis of the inflammatory response to ischemic-reperfusion which is exacerbated in diabetes. Oxidative stress during reperfusion is markedly balanced in diabetes and this appears to results from increased leukocyte recruitment and a higher capacity of diabetic leukocytes to generate ROS in response to stimulation. Several adhesion molecules are expressed on endothelial cells and participate in leukocyte adhesion to the endothelium. These molecules are important for monocyte-endothelium interaction in the initiation and progression of atherosclerosis. The monocyte-macrophage is a pivotal cell in atherogenesis. Cellular adhesion molecules mediate attachment and transmigration of leukocytes across the endothelial surface and are thought to play a crucial role in the early steps of atherogenesis (Seckin et al., 2006). Adhesion molecule VCAM-1 is not expressed under baseline conditions but is rapidly induced by proatherosclerotic conditions in rabbits, mice, and humans, including in early lesions. Initially, it is unclear whether VCAM-1 is simply a marker for atherogenesis or whether it acts in this disease pathway. AGEs promote VCAM-1 expression and atheroma formation in rabbits (Vlassara et al., 1995) and in cultured human endothelial cells (Schmidt et al., 1995). These results suggest the involvement of AGEs in the accelerated coronary atherosclerosis on diabetes (Zhang et al., 2003). Plasma concentrations of VCAM-1 are elevated also in T1DM patients with microalbuminuria and overt nephropathy (Schmidt et al., 1996; Clausen et al., 2000).

Diabetic nephropathy is characterized by specific morphological changes including glomerular basement membrane thickening, mesangial expansion and glomerular and tubulointerstitial sclerosis. The first clinical manifestation of diabetic nephropathy is microalbuminuria, defined as a urinary albumin excretion rate of 20 to 200 microgram/min. Growth factor TGF-beta1 is one of profibrotic cytokines and is important mediator in the pathogenesis of diabetic nephropathy (Goldfarb & Ziyadeh, 2001; Schrijvers et al., 2004; Wang et al., 2005; Wolf & Ziyadeh, 2007). TGF-beta1 stimulates production of extracellular

matrix components such as collagen-IV, fibronectin, proteoglycans (decorin, biglycan). TGF-beta1 may cause glomerulosclerosis and its one of the causal factor in myointimal hyperplasia after balloon injury of carotid artery. It mediates angiotensin-II modulator effect on smooth muscle cell growth. Besides profibrotic activity, TGF-beta1 has immunoregulatory function on adaptive immunity too. AGEs induce connective tissue growth factor-mediated renal fibrosis through TGF-beta1-independent Smad3 signalling (Zhou et al., 2004; Chung et al., 2010).

The present study investigates the relationship between diabetes complications presence, diabetes control (represented by actual levels of HbA1c (HbA1cA) and mean of HbA1c during the last 2 years (HbA1cP)), early glycation products (fructosamine (FAM)), serum advanced glycation end products (s-AGEs), lipid peroxidation products (LPO), advanced oxidation protein products (AOPP), profibrotic cytokines and adhesive molecules in patients with T1DM. We wanted to find a relationship of DC to glycative and oxidative stress parameters, circulating (serum) TGF-beta1 and soluble VCAM-1. Further, we aimed to compare measured parameters in groups -DC, one with DR, with DR combined with another DC and one with only DC another than DR and their combinations. The further aim of the present study was to evaluate if monitoring of circulating FAM, HbA1c, s-AGEs, AOPP, LPO in patients with T1DM could be useful to predict the diabetic complications development.

## 2. Study design and methods

### 2.1 Patients and design

The studied group consisted of 46 children and adolescents with T1DM regularly attending the 1<sup>st</sup> Department of Pediatrics, Children Diabetological Center of the Slovak Republic, University Hospital, Faculty of Medicine, Comenius University, Bratislava. They had T1DM with duration at least for 5 years. One of children was obese (BMIc 97 percentile) and three of them were of overweight (BMIc about 90 percentile). The file was divided into two subgroups: 20 persons without DC (-DC) and 46 those with them (+DC). Then the file of +DC patients was divided into several subgroups according to particular complications: the patients only with retinopathy, those with neuropathy combined with another kinds of DC and those with other than retinopathy to compare the parameters of glycative and oxidative stress and cytokines in each mentioned subgroups. The urine samples in our patients were collected 3 times overnight, microalbuminuria was considered to be positive when UAER was between 20 and 200 microgram/min in 2 samples. No changes (fundus diabetic retinopathy) were found by the ophthalmologist examining the eyes in subject without retinopathy. Diabetic neuropathy was confirmed by EMG exploration using the conductivity assessment of sensor and motor fibres of peripheral nerves. The controls file consists of 26 healthy children. The samples of EDTA capillary blood were used to determine of HbA1c and serum samples were used to determine of FAM, s-AGEs, AOPP and VCAM-1. The samples of serum were stored in -18°C/-80°C.

### 2.2 Parameter analysis

#### 2.2.1 Determination of UAER

UAER was determined by means of immunoturbidimetric assay (Cobas Integra 400 Plus, Roche, Switzerland), using the commercial kit 400/400Plus. The assay was performed as a part of patients routine monitoring in Department of Laboratory Medicine, University Hospital, Bratislava.

### **2.2.2 Determination of fructosamine**

For the determination of fructosamine we used a kinetic, colorimetric assay and subsequently spectrophotometrical determination at wavelength 530 nm. We used 1-deoxy-1-morpholino-fructose (DMF) as the standard. Serum samples were stored at -79°C and were defrost only once. This test is based on the ability of ketoamines to reduce nitroblue tetrazolium (NBT) to a formazan dye under alkaline conditions. The rate of formazan formation, measured at 530 nm, is directly proportional to the fructosamine concentration. Measurements were carried out in one block up to 5 samples. To 3 ml of 0.5 mmol/l NBT were added 150 microliters of serum and the mixture was incubated at 37°C for 10 minutes. The absorbance was measured after 10 min and 15 min of incubation at Novaspec analyzer II, Biotech (Germany).

### **2.2.3 Determination of glycated hemoglobin HbA1c**

HbA1c was determined from EDTA capillary blood immediately after obtained by the low-pressure liquid chromatography (LPLC) (DiaSTAT, USA) in conjunction with gradient elution. Before testing hemolysate is heated at 62-68°C to eliminate unstable fractions and after 5 minutes is introduced into the column. Hemoglobin species elute from the cation-exchange column at different times, depending on their charge, with the application of buffers of increasing ionic strength. The concentration of hemoglobins is measured after elution from the column, which is then used to quantify HbA1c by calculating the area under each peak. Instrument calibration is always carried out when introducing a new column set procedure (Bio-RAD, Inc., 2003).

### **2.2.4 Determination of serum AGEs**

Serum AGEs were determined as AGE-linked specific fluorescence, serum was diluted 20-fold with deionized water, the fluorescence intensity was measured after excitation at 346 nm, at emission 418 nm using a spectrophotometer Perkin Elmer LS-3, USA. Chinine sulphate (1 microgram/ml) was used to calibrate the instrument. Fluorescence was expressed as the relative fluorescence intensity in arbitrary units (A.U.).

### **2.2.5 Determination of serum lipoperoxides**

Serum lipid peroxides were determined by iodine liberation spectrophotometrically at 365 nm (Novaspec II, Pharmacia LKB, Biotech, SRN). The principle of this assay is based on the oxidative activity of lipid peroxides that will convert iodide to iodine. Iodine can then simply be measured by means of a photometer at 365 nm. Calibration curves were obtained using cumene hydroperoxide. A stoichiometric relationship was observed between the amount of organic peroxides assayed and the concentration of I<sub>3</sub> produced (El-Saadani et al., 1989).

### **2.2.6 Determination of serum AOPP**

AOPP were determined in the plasma using the method previously devised by Witko-Sarsat et al. (1996), modified by Kalousova et al. (2002). Briefly, AOPP were measured by spectrophotometry on a reader (FP-901, Chemistry Analyser, Labsystems, Finland) and were calibrated with chloramine-T solutions that in the presence of potassium iodide absorb at 340 nm. In standard wells, 10 microliters of 1.16 M potassium iodide was added to 200



microliters of chloramine-T solution (0–100 micromol/l) followed by 20 microliters of acetic acid. In test wells, 200 microliters of plasma diluted 1:5 in PBS was placed to cell of 9 channels, and 20 microliters of acetic acid was added. The absorbance of the reaction mixture is immediately read at 340 nm on the reader against a blank containing 200 microliters of PBS, 10 microliters of potassium iodide, and 20 microliters of acetic acid. The chloramine-T absorbance at 340 nm being linear within the range of 0 to 100 micromol/l, AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents.

### 2.2.7 Determination of TGF- beta1

Quantitative detection of TGF- beta1 in serum was done by enzyme linked immunosorbent assay, using human TGF-beta1 ELISA-kit (BMS249/2, Bender MedSystem).

Brief description of the method: into washed, with anti-TGF-beta1 precoated microplate were added prediluted (1:10) sera (100 microliters) and “HRP-Conjugate” (50 microliters) as a antihuman-TGF-beta1 monoclonal antibody and incubated for 4 hour on a rotator (100rpm). After microplate washing (3 times) “TMB Substrate Solution” (100 microliters) was added and was incubated for 10 minutes. Enzyme reaction was stopped by adding “Stop Solution” (100 microliters). The absorbance of each microwell was readed by HumaReader spectrophotometer (Human) using 450 nm wavelength. The TGF-beta1 concentration was determined from standard curve prepared from seven TGF-beta1 standard dilutions. Each sample and TGF-beta1 standard dilution were done in duplicate.

### 2.2.8 Determination of serum soluble form of adhesion molecule VCAM-1

For serum soluble form of VCAM-1 (sVCAM-1) estimating we used bead-based multiplex technology and Athena Multi-Lyte™ Luminex 100 xMAP (multi-analyte profiling) analyser. We used RnD systems manufacturer kits: „Human Adhesion Molecule MultiAnalyte Profiling Base Kit“ and „Fluorokine® MAP Human sVCAM-1/CD106 Kit“.

Analyte-specific antibodies are pre-coated onto color-coded microparticles. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest are added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the captured biotinylated antibody, is added to each well. A final wash removes unbound Streptavidin-PE and the microparticles are resuspended in buffer and read using the Luminex analyzer. One laser is microparticle-specific and determines which analyte is being detected. The other laser determines the magnitude of the phycoerythrin-derived signal, which is in direct proportion to the amount of analyte bound (R&D Systems, Inc. 2010).

### 2.2.9 Statistical analysis

Shapiro-Wilk test was performed to the test the distribution of all continuous variables. The variables with normal distribution were compared by one way ANOVA test followed by Bonferroni’s post-test and the results was expressed as mean  $\pm$  SD. Since the evaluated variables did not have normal distribution, we compared them with Kruskal-Wallis non-parametric analysis of variance (ANOVA) followed by Bonferroni’s post-test and the results was expressed as median (1st quartile, 3rd quartile). The Fisher’s test was used to compare the subgroups in regard to diabetic retinopathy and other complications presence/absence. Pearson’s test with correlation coefficient  $r$  or Spearman’s one with Spearman's rank correlation coefficient  $R$  in case of small count of variables were then used to evaluate the

association between parameters described within the text, in all studied patients and in diabetic and non-diabetic subgroups. P values less than 0.05 were accepted as being statistically significant. All statistical analyses were carried out using Excel 2003, Origin 8 and BioSTAT 2009.

### 3. Results

Clinical and biochemical characteristics of the patients with T1DM without and with diabetic complications and controls (CTRL) are reported in Table 1.

	CTRL	n	T1DM -DC	n	T1DM +DC	N
Age (yrs.)	9.0(6.1,14.0)	26	14.4(12.4, 17.9) <sup>ab</sup>	20	16.4(15.1, 17.6) <sup>a</sup>	26
DD (yrs.)	-	0	6(5.5, 8.1) <sup>b</sup>	20	10.0(7.9, 12.9)	26
HbA1cA (%)	5.0 ± 0.3	18	8.3 ± 1.4 <sup>ab</sup>	20	10.4 ± 1.4 <sup>a</sup>	26
FAM (mmol/l)	1.67 ± 0.31	24	2.64 ± 0.38 <sup>ab</sup>	20	3.06 ± 0.48 <sup>a</sup>	26
s-AGEs (A.U.)	54.9 ± 9.9	22	64.4 ± 10.1 <sup>ab</sup>	20	71.8 ± 11.6	24
AOPP (micromol/l)	58.8(52.0, 71.8)	11	43.3(42.6, 60.4)	17	78.2(49.5, 114.6)	20
LPO (nmol/ml)	100(88, 110)	10	106(105, 161)	19	127(109, 152) <sup>a</sup>	17
TGF-beta1 (ng/ml)	3.30 ± 3.41	8	5.9 ± 4.14 <sup>b</sup>	10	10.49 ± 4.55 <sup>a</sup>	16
VCAM-1 (ng/ml)	12.6 ± 3.7	15	17.1 ± 3.1	19	17.4 ± 3.3 <sup>a</sup>	26

<sup>a</sup> significant difference in comparison with CTRL

<sup>b</sup> significant difference in comparison with +DC group T1DM

Table 1. Clinical and biochemical characteristics of the patients with T1DM and controls

As seen, HbA1c and FAM were significantly elevated in both diabetic groups in comparison with controls and also in +DC vs. -DC those. Serum AGEs were significantly elevated in +DC compared to -DC and also to controls, but the difference between -DC and controls was not significant. The levels of AOPP were evidently higher in +DC compared to controls, but the difference was not significant. The levels of LPO were significantly elevated in +DC vs. controls, the differences between both diabetic groups and between -DC vs. controls were not significant. The levels of TGF-beta1 similarly to s-AGEs were significantly elevated in +DC compared to -DC and also to controls, but the difference between -DC and controls was not significant (Fig. 1). In terms of the VCAM-1 values, only between +DC and controls there were found significant difference there (Fig. 2).

The levels of TGF-beta1 are significantly elevated in +DC compared to -DC ( $10.49 \pm 4.55$  vs.  $5.9 \pm 4.14$  ng/ml,  $p < 0.05$ ) and also to controls ( $10.49 \pm 4.55$  vs.  $3.30 \pm 3.41$  ng/ml,  $p < 0.05$ ), but the difference between -DC and controls ( $5.9 \pm 4.14$  vs.  $3.30 \pm 3.41$  ng/ml,  $p > 0.05$ ) was not statistically significant.

#### 3.1 The relationships between clinical and biochemical parameters

##### 3.1.1 The subgroup of patients with T1DM without diabetic complications

The relationships characterized by Pearson's correlation coefficient  $r$  or Spearman's coefficient  $R$  between the parameters described within the text are reported in Table 2. As seen, we found significant linear correlations of FAM with HbA1cA ( $r=0.676$ ), LPO with HbA1cP ( $r=-0.507$ ) and AOPP ( $R=0.671$ ). The relationship between LPO (y) and HbA1cP(x) is possible to describe by non-linear equation  $y=19x^2-354x+1752$  ( $R=0.632$ ,  $R^2=0.400$ ,  $p < 0.05$ ). VCAM-1 significantly correlated with age ( $r=-0.478$ ), HbA1cA ( $r=0.653$ , Fig. 3), HbA1cP ( $r=0.501$ ) and with FAM ( $r=0.630$ , Fig. 4).

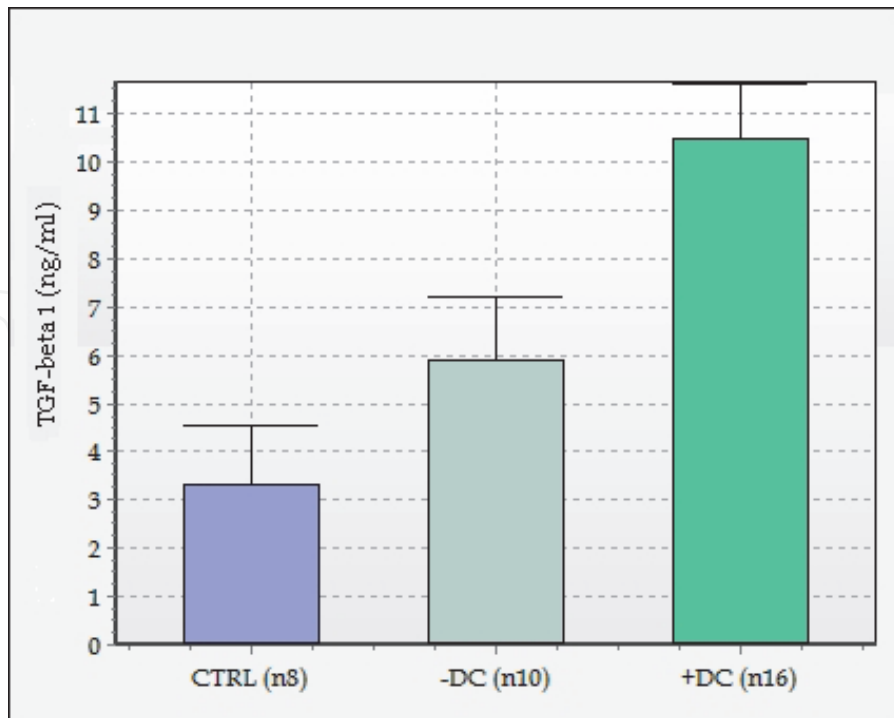


Fig. 1. Comparison of TGF-beta1 levels of patients with T1DM and controls

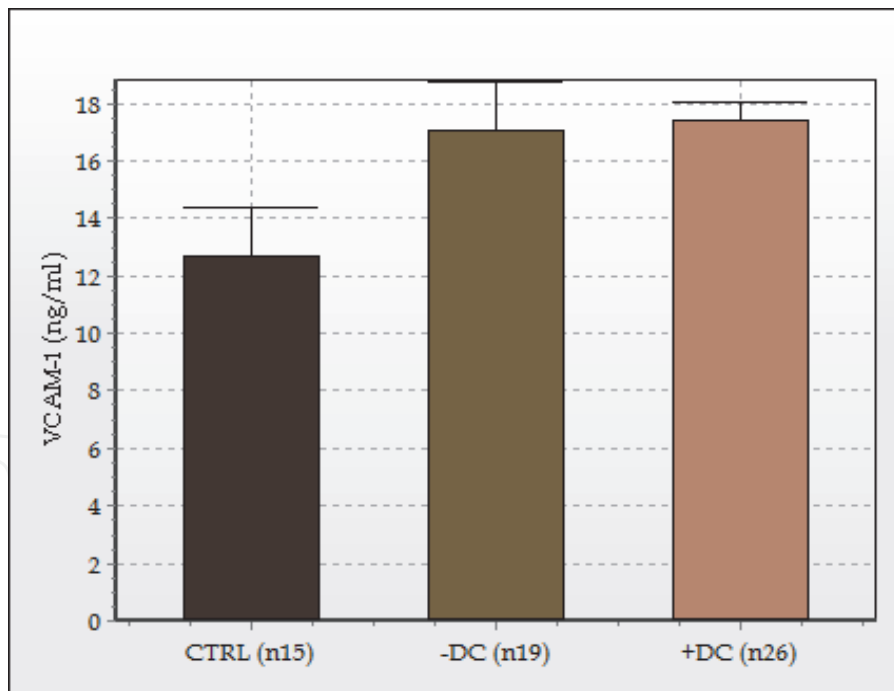


Fig. 2. Comparison of VCAM-1 levels of patients with T1DM and controls

The levels of VCAM-1 are significantly elevated in +DC compared to controls ( $17.4 \pm 3.3$  vs.  $12.6 \pm 3.7$  ng/ml,  $p < 0.05$ ). The values of VCAM-1 in -DC subgroup differ obviously from those in controls, but the difference is non statistically significant ( $17.1 \pm 3.1$  vs.  $12.6 \pm 3.7$  ng/ml,  $p > 0.05$ ). There are similar levels in both diabetic subgroups ( $17.4 \pm 3.3$  vs.  $17.1 \pm 3.1$  ng/ml,  $p >> 0.05$ ).



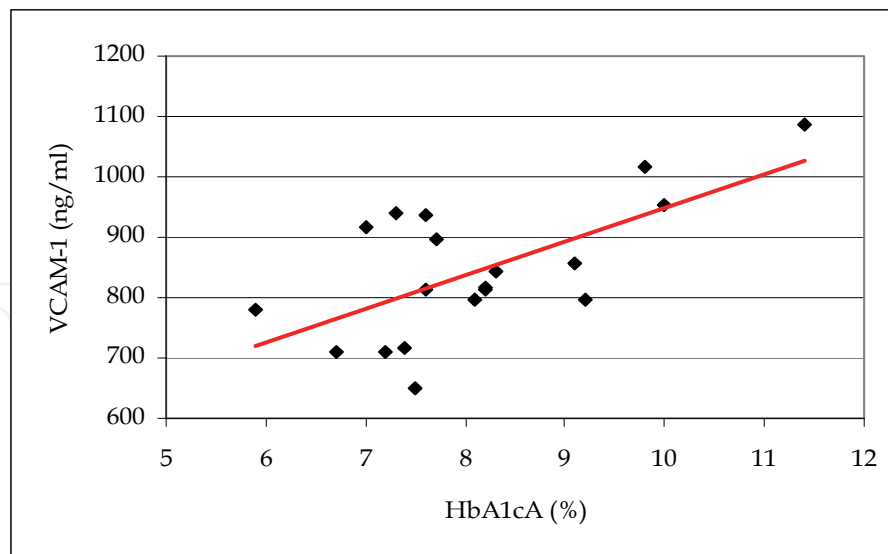


Fig. 3. The linear correlation between VCAM-1 and HbA1cA

	DD (yrs.)	HbA1cA	HbA1cP	FAM	s-AGEs	AOPP	LPO	TGF-beta1	VCAM-1
Age (yrs.)	0.205	-0.267	-0.232	-0.328	-0.030	0.130 <sup>†</sup>	0.334	0.406 <sup>†</sup>	-0.478 <sup>Δ</sup>
DD (yrs.)	N	0.152	-0.008	0.326	-0.256	-0.193 <sup>†</sup>	-0.001	0.036 <sup>†</sup>	0.089
HbA1cA (%)		N	0.748 <sup>Δ</sup>	0.676 <sup>Δ</sup>	0.217	-0.298 <sup>†</sup>	-0.398	-0.588 <sup>†</sup>	0.653 <sup>Δ</sup>
HbA1cP (%)			N	0.433	0.302	-0.400 <sup>†</sup>	-0.507 <sup>Δ</sup>	-0.042 <sup>†</sup>	0.501 <sup>Δ</sup>
FAM (mmol/l)				N	0.134	0.081 <sup>†</sup>	-0.260	-0.224 <sup>†</sup>	0.630 <sup>Δ</sup>
s-AGEs (A.U.)					N	0.197 <sup>†</sup>	0.270	0.006	0.068
AOPP (micromol/l)						N	0.671 <sup>†Δ</sup>	0.477 <sup>†</sup>	-0.447 <sup>†</sup>
LPO (nmol/ml)							N	0.200 <sup>†</sup>	-0.404
TGF-beta1 (ng/ml)								N	-0.578 <sup>†</sup>

Table 2. The relationships between the parameters in patients with T1DM without diabetic complications (<sup>†</sup> R, <sup>Δ</sup> p<0.05)

In this subgroup LPO and VCAM-1 were in association also with other parameters, but those were not statistically significant. Non linear statistically significant relationship with regression line equation  $y=0.33x^2 - 4.10x + 28.26$  was found between VCAM-1(y) and HbA1cA(x) ( $R=0.694$ ,  $R^2=0.481$ ,  $p<<0.05$ ).

### 3.1.2 The subgroup of patients with T1DM with diabetic complications

The relationships characterized by Pearson's correlation coefficient  $r$  or Spearman's coefficient  $R$  between the parameters described within the text are reported in Table 3.

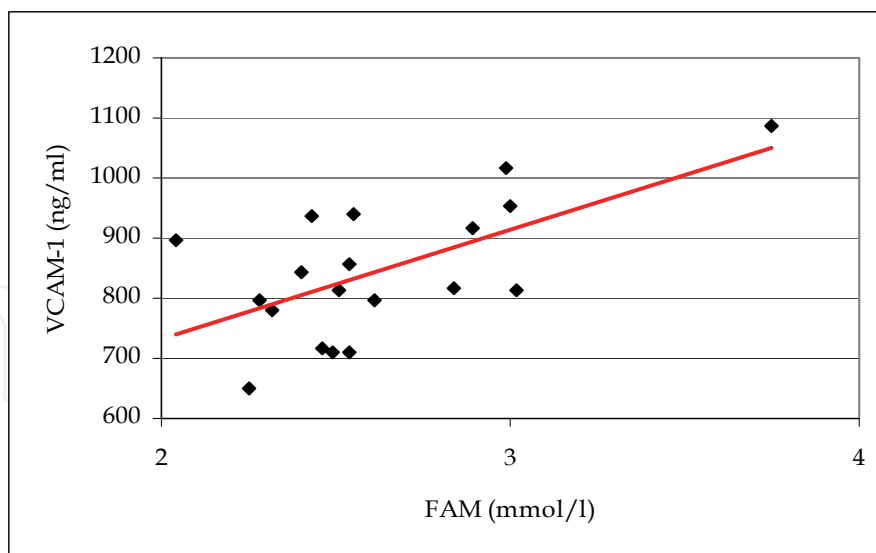


Fig. 4. The linear correlation between VCAM-1 and FAM

	DD (yrs.)	HbA1cA	HbA1cP	FAM	s-AGEs	AOPP	LPO	TGF-beta1	VCAM-1
Age (yrs.)	0.448 <sup>Δ</sup>	-0.295	-0.422 <sup>Δ</sup>	-0.023	-0.068	-0.165	-0.044 <sup>†</sup>	0.541 <sup>†Δ</sup>	0.067
DD (yrs.)	N	-0.070	-0.110	-0.170	-0.069	-0.034	-0.297 <sup>†</sup>	0.247 <sup>†</sup>	0.009
HbA1cA (%)		N	0.539 <sup>Δ</sup>	0.581 <sup>Δ</sup>	0.221	0.278	0.123 <sup>†</sup>	-0.429 <sup>†</sup>	0.006
HbA1cP (%)			N	0.3405	0.247	0.116	0.127 <sup>†</sup>	-0.679 <sup>†Δ</sup>	0.291
FAM (mmol/l)				N	0.479 <sup>Δ</sup>	0.538 <sup>Δ</sup>	0.471 <sup>†</sup>	-0.708 <sup>†Δ</sup>	0.183
s-AGEs (A.U.)					N	0.119 <sup>†</sup>	0.125 <sup>†</sup>	-0.356 <sup>†</sup>	0.432 <sup>Δ</sup>
AOPP (micromol/l)						N	0.355 <sup>†</sup>	-0.545 <sup>†</sup>	-0.026
LPO (nmol/ml)							N	-0.612 <sup>†</sup>	-0.174 <sup>†</sup>
TGF-beta1 (ng/ml)								N	-0.069 <sup>†</sup>

(<sup>†</sup>R, <sup>Δ</sup>p<0.05)

Table 3. The relationships between parameters described within the text in patients with T1DM with diabetic complications

As seen, in +DC subgroup we found significant correlations of FAM with HbA1cA (r=0.581), s-AGEs with FAM (r=0.479) and AOPP with FAM (r=0.538). LPO correlated with FAM (r=0.471), but this relation is not statistically significant (p=0.056). TGF-beta1 correlated with age (R=0.541), HbA1cP (R=-0.679) and FAM (R=-0.708). Statistically significant moderate linear correlation was found between VCAM-1 and s-AGEs (r=0.432). Moderate relationships were found also between TGF-beta1 and oxidative stress parameters, but those were not statistically significant.

### 3.1.3 Controls

As seen in Table 4, there were found moderate negative relation on the border of significance between AOPP and FAM ( $R=-0.627$ ,  $p=0.05$ ) and strong relation between AOPP and s-AGEs ( $R=0.855$ ) in controls. TGF-beta1 was in statistically significant relation with age ( $R=-0.838$ ) and s-AGEs ( $R=-0.757$ ) and moderate, but not significant relationship was found with LPO ( $R=0.478$ ). Slight relationship were found between VCAM-1 and FAM ( $R=0.366$ ) and also between VCAM-1 and s-AGEs ( $R=0.267$ ).

	HbA1cA	FAM	s-AGEs	AOPP	LPO	TGF-beta1	VCAM-1
Age (yrs.)	0.354 <sup>†</sup>	-0.249	-0.008	0.193 <sup>†</sup>	0.026 <sup>†</sup>	-0.838 <sup>†Δ</sup>	-0.052 <sup>†</sup>
HbA1cA (%)	N	0.109 <sup>†</sup>	-0.133 <sup>†</sup>	-0.022 <sup>†</sup>	0.189 <sup>†</sup>	-0.024 <sup>†</sup>	-0.068 <sup>†</sup>
FAM (mmol/l)		N	-0.143	-0.627 <sup>†■</sup>	-0.162 <sup>†</sup>	0.276 <sup>†</sup>	0.366 <sup>†</sup>
s-AGEs (A.U.)			N	0.855 <sup>†Δ</sup>	-0.382 <sup>†</sup>	-0.757 <sup>†Δ</sup>	0.267 <sup>†</sup>
AOPP (micromol/l)				N	-0.286 <sup>†</sup>	N	0.069 <sup>†</sup>
LPO (nmol/ml)					N	0.478 <sup>†</sup>	0.037 <sup>†</sup>
TGF-beta1 (ng/ml)						N	0.152 <sup>†</sup>

(<sup>†</sup>R,  $p<0.05$ , <sup>■</sup>  $p=0.05$ )

Table 4. The relationships between the parameters in controls

### 3.2 The parameters of glycativ and oxidative stress, TGF-beta1 and VCAM-1 with regard to presence/absence of retinopathy and/or other complications

We compared described parameters between subgroups with/without diabetic retinopathy. The results of Fisher's post-test ( $p$  values) are reported in table 5.

Subgroups	FAM	HbA1cA	s-AGEs	AOPP	LPO	TGF-beta1	VCAM-1
DR vs. DR+O	NS	NS	NS	NS	NS	NA	NS
DR vs. ODC	0.055	NS	0.055	NS	<0.05	<0.05	NS
DR vs. -DC	NS	0,01	NS	NS	NS	<0.05	NS
DR+O vs. ODC	0.052	NS	0,05	NS	NS	NA	NS
DR+O vs. -DC	NS	<0.05	NS	NS	NS	NA	NS
ODC vs. -DC	<0.05	<0.05	<0.05	NS	NS	NS	NS

(DR - having diabetic retinopathy only, DR+ODC - having diabetic retinopathy and another complications, ODC - having only other diabetic complications except diabetic retinopathy-DC - having no complications, NS - non-significant difference, NA - not available)

Table 5. The differences in measured parameters between subgroups of patients with T1DM with regard to presence/absence of diabetic retinopathy and/or other (O) complications

FAM were significantly elevated in patients having diabetic complications only other than diabetic retinopathy compared to -DC (3.10(2.93, 3.54) vs. 2.54(2.42, 2.91) mmol/l,  $p < 0.05$ , Fig. 5). HbA1c levels are elevated in patients having diabetic retinopathy against to -DC (9.8(9.6, 10.2) vs. (7.9(7.4, 9.1)%,  $p < 0.05$ ), in subgroup of patients having diabetic retinopathy with other complication/s compared to -DC (10.4(8.6, 11.2) vs. (7.9(7.4, 9.1) %,  $p < 0.05$ , Fig.6) and also the subgroup of patients having diabetic complications only other than diabetic retinopathy compared to -DC (10.5(10.0, 11.1 vs. (7.9(7.4, 9.1) %,  $p < 0.05$ , Fig. 6). Serum AGEs were significantly higher in subgroup with only other diabetic complications than diabetic retinopathy compared to -DC one (74.8(71.2, 76.5) vs. 61.9(58.9, 71.0) A.U., Fig. 7), and non-significantly higher in patients with retinopathy only than in those with others DC and also in patients with DR and another DC compared to ODC group, however, p-values were only slightly higher than 0.05 (Fig. 7). The values of LPO were significantly elevated in patients with complications other than retinopathy compared to those with retinopathy only (138(129, 165) vs. 101(93, 109) nmol/ml, Fig. 8). No significant differences were found between others in LPO. There were the significant differences between patients having only diabetic retinopathy vs. -DC in TGF-beta1 levels (14.17(13.32, 15.52) vs. 5.7(2.23, 8.71) ng/ml,  $p < 0.05$ , Fig. 9) and also between subgroup of patients having only diabetic retinopathy and those having diabetic complications other than diabetic retinopathy (14.17(13.32, 15.52) vs. 9.05(5.29, 10.39) ng/ml,  $p = 0.05$ , Fig. 9). Neither AOPP parameters nor VCAM-1 showed any significant differences between subgroups with regard to presence/absence diabetic retinopathy or other diabetic complications.

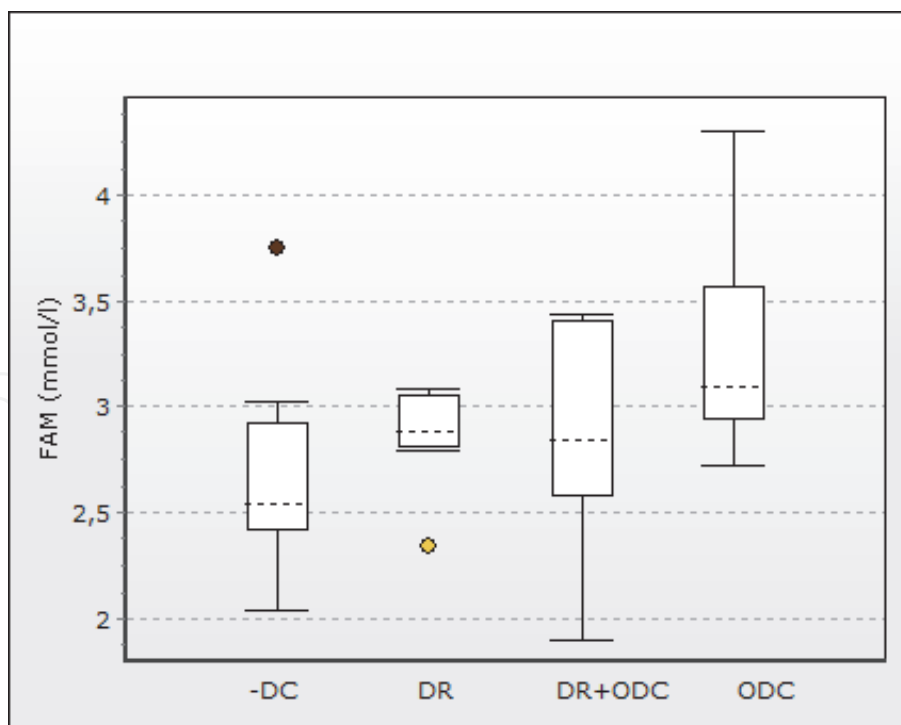


Fig. 5. The values of FAM in subgroups of patients with T1DM with regard to diabetic retinopathy presence/absence (DR - having diabetic retinopathy only, DR+ODC - having diabetic retinopathy and another complications, ODC - having only other diabetic complications except diabetic retinopathy, -DC - having no complications)

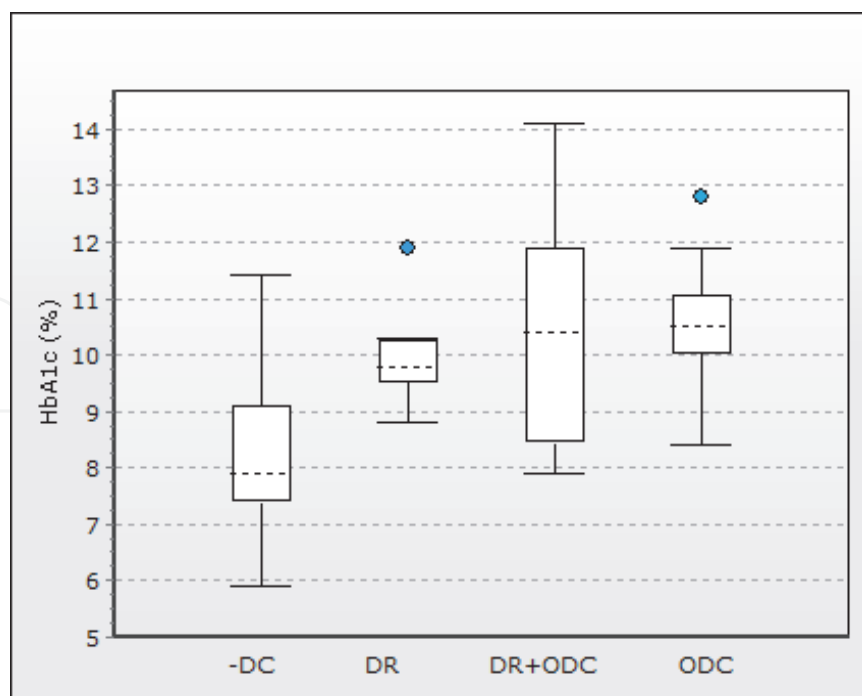


Fig. 6. The values of HbA1c in subgroups of patients with T1DM with regard to diabetic retinopathy presence/absence (DR - having diabetic retinopathy only, DR+ODC - having diabetic retinopathy and another complications, ODC - having only other diabetic complications except diabetic retinopathy, -DC - having no complications)

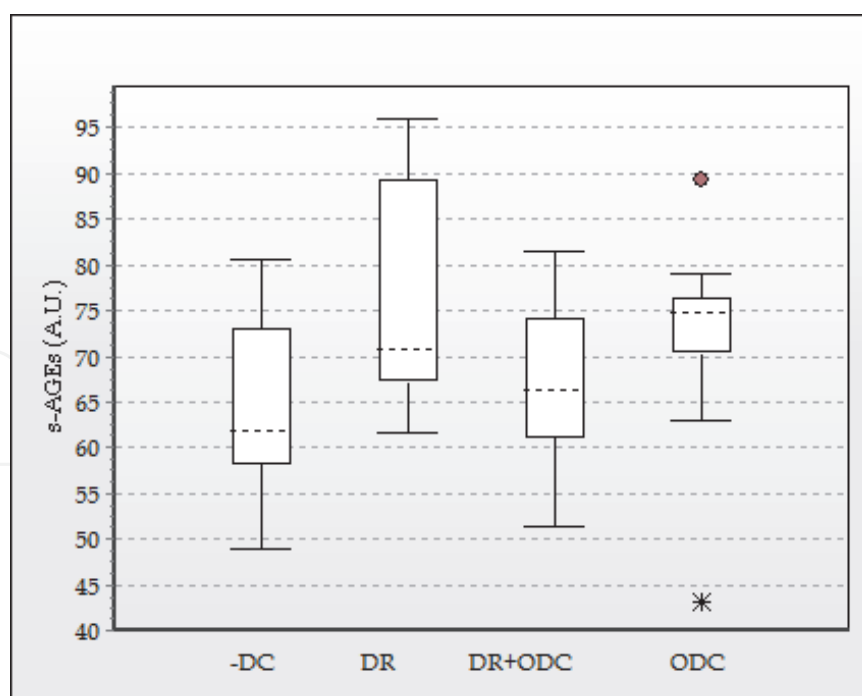


Fig. 7. The values of s-AGEs in subgroups of patients with T1DM with regard to diabetic retinopathy presence/absence (DR - having diabetic retinopathy only, DR+ODC - having diabetic retinopathy and another complications, ODC - having only other diabetic complications except diabetic retinopathy, -DC - having no complications)



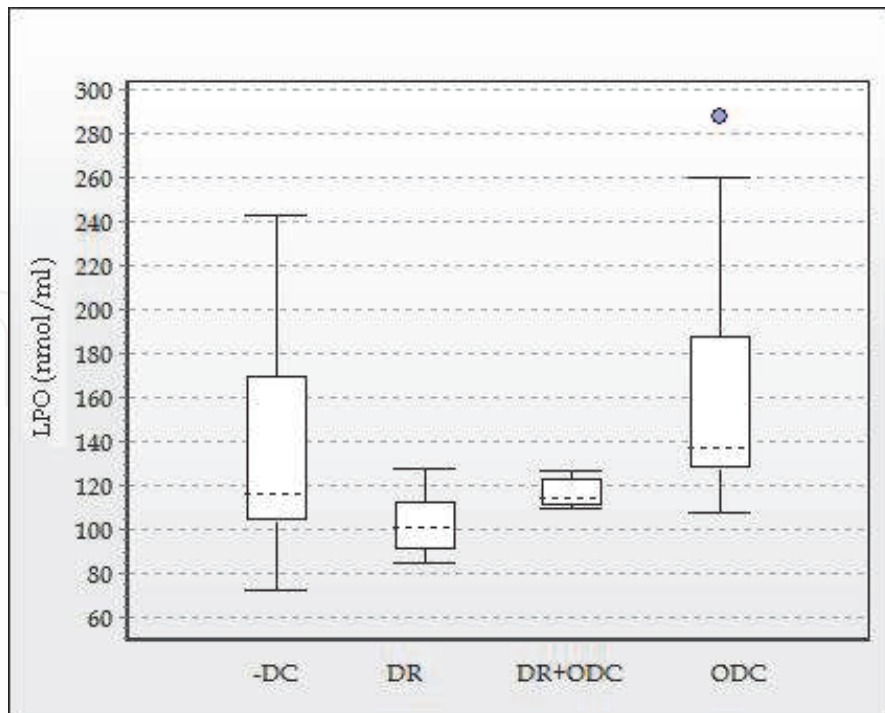


Fig. 8. The values of LPO in subgroups of patients with T1DM with regard to diabetic retinopathy presence/absence (DR - having diabetic retinopathy only, DR+ODC - having diabetic retinopathy and another complications, ODC - having only other diabetic complications except diabetic retinopathy, -DC - having no complications)

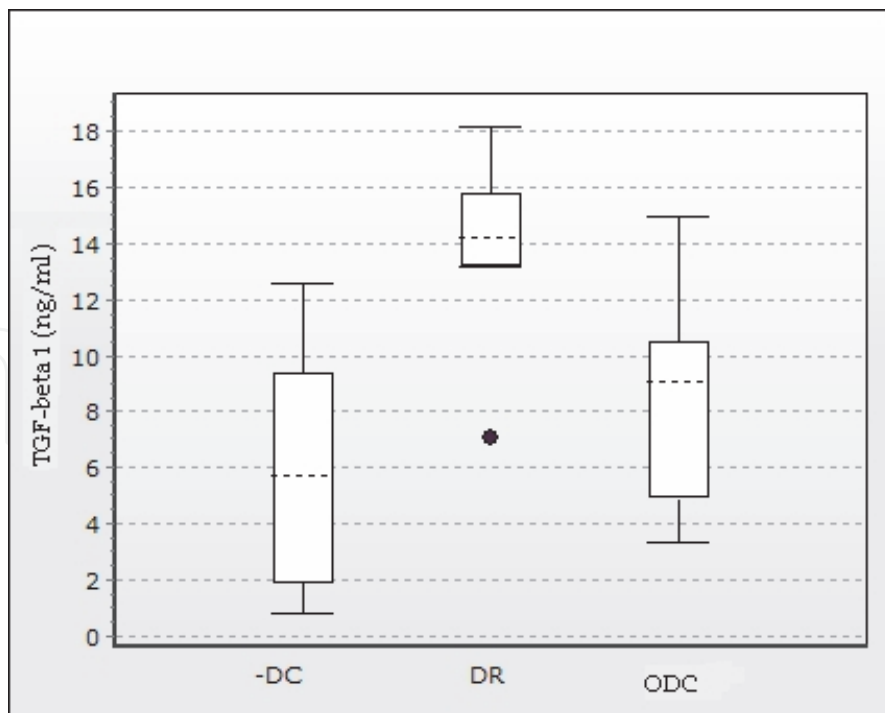


Fig. 9. The values of TGF-beta1 in subgroups of patients with T1DM with regard to diabetic retinopathy presence/absence (DR - having diabetic retinopathy only, ODC - having only other diabetic complications except diabetic retinopathy, -DC - having no complications)

#### 4. Conclusion

Our results suggest the relation of glycation and oxidation to profibrotic cytokines, vascular molecules and diabetic complications. Serum AGEs were connected with complications other than retinopathy more than just with retinopathy, nevertheless, some relation of retinopathy and s-AGEs was found (p-values were only slightly higher than 0.05). Lipoperoxides showed some relation to DR since higher in patients with retinopathy than in those with other DC, whereas AOPP did not show any relation to any DC. It seems that in our patients TGF-beta1 and VCAM-1 are linked with the development of DC, but only TGF-beta1 showed some linkage to diabetic retinopathy.

We ought to keep in mind the fact our investigation concerns the children and adolescents. Maybe the study of older patients with T1DM would show more, especially about VCAM-1 and its relation to glycative and oxidative stress and consequently to development of retinopathy/other complications.

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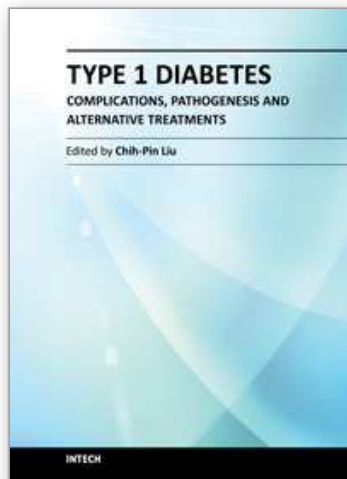
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This book is intended as an overview of recent progress in type 1 diabetes research worldwide, with a focus on different research areas relevant to this disease. These include: diabetes mellitus and complications, psychological aspects of diabetes, perspectives of diabetes pathogenesis, identification and monitoring of diabetes mellitus, and alternative treatments for diabetes. In preparing this book, leading investigators from several countries in these five different categories were invited to contribute a chapter to this book. We have striven for a coherent presentation of concepts based on experiments and observation from the authors own research and from existing published reports. Therefore, the materials presented in this book are expected to be up to date in each research area. While there is no doubt that this book may have omitted some important findings in diabetes field, we hope the information included in this book will be useful for both basic science and clinical investigators. We also hope that diabetes patients and their family will benefit from reading the chapters in this book.

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