Angiogenic factors in Children and Adolescents with Type 1 Diabetes Mellitus

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Abstract: Background: Data on angiogenesis in pediatric patients with type 1 diabetes mellitus (T1DM) are scarce. **Objective:** The aim of this cross sectional study was to determine circulating levels of angiogenic factors [angiopoietin (Ang)-2, vascular endothelial growth factor (VEGF), adrenomedullin (AM)] in children and adolescent with T1DM without vascular complications. Additionally, to investigate whether these angiogenic parameters were associated with metabolic control and disease duration. **Methods:** This study included 50 diabetic children and adolescents (mean age 11.04±2.65 years) and forty healthy subjects (mean age 10.50±2.09 years) matched with patient's age-and sex as control group. Patients and controls were assessed for glycosylated hemoglobin (HbA1c) and plasma Ang-2, VEGF, AM assay using by enzyme-linked immunosorbent assay. **Results:** In T1DM patients, positive family history was found in 26 (52.00%) and 33 (66.00%) patients were had poor metabolic control. In T1DM patients, HbA1c, glucose, insulin, VEGF were significantly increased (P <0.0001 for all) while Ang-2 was significantly decreased (P <0.001) than controls. In T1DM with short (<5 years) and T1DM with long duration (≥ 5years), HbA1c, glucose, insulin, VEGF were significantly increased (P <0.0001 for all) while Ang-2 was significantly decreased (P <0.003, P <0.011) compared with healthy control. Serum glucose was significantly higher in patients with T1DM with long duration versus those with short duration (P <0.034). In T1DM patients, positive correlations were found between HbA1c with VEGF (r= 0.266; P <0.031), glucose (r= 0.670; P <0.0001); between glucose with VEGF (r= 0.258; P <0.035); between adrenomedullin with Ang-2 (r= 0.434; P <0.001). Meanwhile, a significant negative correlation was found between adrenomedullin and insulin (r= -0.235; P <0.038). **Conclusion:** The results of our study enlighten the behavior of 3 different angiogenic factors (VEGF, Ang-2, and adrenomedullin) in pediatric patients with uncomplicated T1DM in which plasma levels VEGF were increased, Ang-2 were decreased and AM were unchanged. Pathophysiology and clinical applications of these findings need further studies.

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1. Introduction:

Type 1 diabetes mellitus (T1DM) is an autoimmune disease resulting from pancreatic β-cell destruction by effector lymphocytes, leading to the loss of insulin production and hyperglycemia. Vascular dysfunction both micro- and macroangiopathy plays a major role in many complications associated with diabetes. Large prospective clinical studies show a strong relationship between hyperglycaemia and diabetic microvascular complications in both T1DM and type 2 diabetes mellitus (T2DM) (1, 2).

Disequilibrium of angiogenesis promoters and inhibitors in diabetes may lead to exuberant but dysfunctional neovascularization. Angiopoietins are growth factors that promote angiogenesis together with vascular endothelial growth factor (VEGF). Among the four identified angiopoietins, angiopoietin (Ang)-1 and Ang-2 are reported to be required for the formation of mature blood vessels, as demonstrated by mouse knock out studies (3). Both Ang-1 and Ang-2 act by binding to the endothelium-specific receptor tyrosine kinase 2 (Tie-2). Angiopoietin-1 mediates vascular remodeling and plays a role in the recruitment of vascular smooth muscle cells and pericytes (4). Angiopoietin-2, as an antagonist for Ang-1, inhibits Ang-1-promoted Tie2 signaling and decreases blood vessel maturation and stabilization. The function of Ang-2 appears to be highly dependent on the presence of VEGF. For example, increased levels of Ang-2 in the presence of low concentrations of VEGF were shown to result in endothelial cell death and vessel regression, whereas in the presence of high levels of VEGF there is increased proliferation and stimulation of angiogenesis (5). The changes of circulating level of Ang-2 in T1DM patients have not been investigated.

VEGF is a member of a family of closely related cytokines, also known as vascular permeability factor. It is highly specific mitogen for vascular

endothelial cells by binding to two tyrosine kinase receptors which are selectively expressed on endothelial cells; it induces endothelial cell proliferation, promotes cell migration and inhibits apoptosis (6). The VEGF is the strongest inducer of both physiological and pathological angiogenesis (7). Apart from glycosylated hemoglobin (HbA1c), which is a well-known risk factor for micro- and macrovascular complications, data suggest that growth factors, including VEGF, may have an important function in the modification of tissue damage and its acceleration (7,8). In earlier studies, it has documented the significance of VEGF in non-proliferative retinopathy development in children and adolescents with T1DM (9,10). Others have also shown the up regulation of VEGF mRNA expression in pericytes as well as podocytes and mesangial cells in the early stages of disease, indicating its function in diabetic angiopathy (11). VEGF has an effect on the growth and maintenance of blood vessels, and Ang-2 induces vascular destabilization and vessel sprouting in the presence of VEGF. Angiopoietin-induced biological effects are dependent both on the cellular context and on the VEGF levels (12). There are no studies about the levels of soluble Ang-2 in any pediatric T1DM without complications.

Adrenomedullin (AM), a ubiquitous regulatory peptide with different actions, is widely synthesized and secreted from most of the cells in the body (13). It controls proliferation, differentiation and migration of cells (14). It has vasodilator and blood pressure lowering properties and plays important role in maintaining electrolyte and fluid homeostasis (15). Moreover, evidence that AM possesses a clear cut proangiogenic effect under both physiological and pathophysiological conditions has accumulated (16). Adrenomedullin is involved in insulin regulatory system and is elevated in plasma from patients with pancreatic dysfunctions such as T1DM, T2DM and insulinoma (17). Adrenomedullin might play a role in the pathogenesis of diabetic vasculopathy in T1DM (18) and T2DM (19).

The current cross sectional study was designed to determine circulating levels of angiogenic factors (angiopoietin-2, vascular endothelial growth factor, adrenomedullin) in children and adolescent with T1DM without vascular complications. To investigate whether these angiogenic parameters were associated with glycemic control and duration of the disease. These may contribute to the development of new therapeutic approaches for prevention and treatment of diabetic vascular complications.

2.Methods:

Participants

Fifty children and adolescents (24 males and 26 females) with T1DM (mean age 10.04, range 6.00–16.50 years) attending the Pediatric Clinic of the King Abdulaziz University Hospital, Jeddah, Saudi Arabia from January 2012-January 2013, were invited, through their parents, to participate in this cross section study. The patients were subdivided according to duration of disease into 2 groups, T1DM with long duration (≥ 5years) and T1DM with short duration (< 5years). T1DM was defined in accordance with the criteria of the American Diabetes Association (20). Forty apparently healthy children and adolescent matched with patient's age (mean age 10.50, range 6.00–13.00 years) and sex (20 males and 20 females) was recruited from outpatients' clinics and considered as controls. Inclusion criteria of patients consisted of: (i) first diagnosis of T1DM made before 18 years of age; (ii) no evidence of diabetic retinopathy (assessed via fundus examination), neuropathy (assessed using clinical history and physical examinations), or nephropathy (determined using 24-hrs urine albumin >30 mg/d); or macrovascular complications (iii) no intake of medications in the preceding 6 months other than insulin; (iv) no history of other chronic diseases including autoimmune diseases. The control subjects do not have any health problems, no family history of diabetes and are not receiving any medications or dietary supplements. All the patients were treated with humanized insulin therapy (0.87 ±0.24 IU of insulin per day/kg of body weight).

All participants were subjected to careful history taking laying stress on onset, duration, frequency of diabetic ketoacidosis (DKA) or hyperglycemic attacks, thorough clinical examination with special emphasis on signs of diabetic complications. Height, weight and body mass index (BMI) were measured and recorded. Fundus examination was performed by an ophthalmologist after maximum papillary dilatation using indirect ophthalmoscope to identify diabetic retinopathic changes.

Written informed consent was obtained from all children and adolescents participating in the study, or from their parent or guardian. This study was approved by The Ethics Committee of the Medical University of King Abdulaziz and the investigation was carried out in accordance with the principles of the Declaration of Helsinki as revised in 1996.

Measurements

Children with T1DM had blood collected for hematology and biochemistry measurements at the time of their routine clinic visit, or in the case of newly diagnosed patients, at least 2 days after admission for initial management and stabilization of diabetes. Three ml of fasting venous blood samples

obtained after 30 min of supine rest from an antecubital vein before the morning injection of insulin (8:00–9:00 a.m.) were collected on EDTA tube, centrifuged at 3.000g for 15 minutes and plasma samples were stored at -70°C till assay. Importantly, we used plasma samples in contrast to many earlier studies, which have used serum samples. We wanted to avoid the production of angiogenic growth factors by the platelets during the segregation of serum from blood sample. Circulating levels of HbA1c, a clinical indicator of blood glucose control, was measured by Hitachi 911 autoanalyzer (Hitachi Co. Ltd., Tokyo, Japan). HbA1c determination is based on turbidimetric inhibition immunoassay for hemolyzed whole blood from Roche/Hitachi 911, Tokyo, Japan. Normal values of HbA1c according to previous report (21) ranged from 4.0 to 6.0%. HbA1c values were recorded for the previous 12-month period from the participants' clinic record and then averaged. Poor metabolic control was considered when HbA1c reached >8.0% (22). Microalbuminuria was assayed using SERAPAK immuno-microalbumin Kit (Bayer Corporation, Benedict Ave, Tarry town, NY, USA). Microalbuminuria was diagnosed when first morning urine sample albumin/creatinine ratio was between 30 and 300 mg/g, in two separate specimens, excluding urinary infection (23). Fasting glucose was measured by enzymatic test (Roche Diagnostics GmbH, Mannheim, Germany). Plasma levels of insulin, VEGF, angiopoietin-2, adrenomedullin were measured by the ELISA method [Immunospec, Canoga Park, CA, USA (insulin); Quantikine High Sensitivity Human by R&D System, Minneapolis, Minn., USA (VEGF); Weka Med Supplies Corp, Th Ave, NY, USA (angiopoietin-2 and adrenomedullin)] according to manufacturer protocols. Minimum detectable concentrations were determined by the manufacturer as 2 µIU/ml (insulin); 5.0 pg/ml (VEGF); 3 ng/L (angiopoietin-2) and 2 ng/L (adrenomedullin). Other routine biochemical tests were performed using standard methodology in clinical pathology laboratory.

Statistical analysis

Statistical Science for Social Package (SPSS V12, SPSS Inc., Chicago, IL, USA) was used for data analysis. Data were presented as mean (SD), minimum – maximum or number (%) as appropriate. For comparison of two groups, the parametric 'Student's *t* test' and non-parametric 'Chi Squared test' for independent variables were used, while comparisons of multiple groups were performed using analysis of variance (ANOVA) and Kruskall Wallis tests for parametric and non-parametric variables, respectively. Spearman's and Pearson's correlation tests were used for correlating non-parametric and parametric variables. For all tests, a probability (*P*) <0.05 was considered significant.

3. Results:

Table (1) showed that there was no significant difference between patients and controls regarding, age, gender and BMI (*P* =0.289, *P* =0.509, *P* =0.543, respectively). Meanwhile, the weight and height were significantly higher in patients versus controls (*P* =0.024 and *P* =0.040).

In the patients, positive family history was found in 26 (52.00%) and 33 (66.00%) patients were had poor glycemic control HbA1c >8%. The duration of the disease was <5 year in 40 (80.00%) patients and ≥ 5 year in 10 (20.00%) patients. The most common presentation was polyuria in 41 (8200%), then polydipsia in 41 (82.00%), abdominal pain 35 (70.00%), diabetic ketoacidosis 27 (54.00%), vomiting 16 (32.00%), septicemia 4 (8.00%), gingivitis 3 (6.00%), valvovaginitis 2 (4.00%) and urinary tract infection 1 (2.00%). The hospital stay range from 3 -1 5 days (Table 2).

In T1DM patients, HbA1c, glucose, insulin, VEGF were significantly increased (*P* <0.0001) while Ang-2 was significantly decreased (*P* <0.001) than controls (Table 3).

In T1DM with short (<5 years) and T1DM with long duration (≥ 5years), HbA1c, glucose, insulin, VEGF were significantly increased (*P* <0.0001 for all) while Ang-2 was significantly decreased (*P* <0.003, *P* <0.011) compared with healthy control. Serum glucose was significantly higher in patients with T1DM with long duration versus those with short duration (*P* <0.034) (Table 4).

In T1DM patients, positive correlations were found between HbA1c with VEGF (r= 0.266; *P* <0.031), glucose (r= 0.670; *P* <0.0001); between glucose with VEGF (r= 0.258; *P* <0.035); adrenomedullin with Ang-2 (r= 0.434; *P* <0.001). Meanwhile, a significant negative correlation was found between adrenomedullin and insulin (r= -0.235; *P* <0.038) (Table 5).

4.Discussion:

VEGF and angiopoietins are the essential regulatory molecules cooperating in induction of blood vessels growth and regression. Dysregulation of angiogenesis in diabetes might be present only in non-compensated patients with severely affected metabolic status (24). Ang-2 may have a role in facilitating VEGF-initiated neovascular sprouting by impairing Ang-1 stabilization and maintenance of existing tubes (25).

In this study, plasma level of Ang-2 was significantly lower in all T1DM patients and also in

T1DM with long and short duration compared with healthy control. All T1DM patients did not have any microvascular or macrovascular complications. It is well established that Ang-2 modulates endothelial cell biology and destabilizes blood vessels to facilitate angiogenesis. Ang-2 is a key angiogenic hypoxia-induced growth factor (26). In this respect, it has been reported that increased levels of Ang-2 have been implicated in the development of diabetic microvascular complications including retinopathy (27). In contrary to our results, others reported that Ang-2 levels are increased in diabetics, particularly in patients with type 2 diabetes compared with controls (28,29), with the highest levels among patients with grade 2 and 3 retinopathy. Diabetic mice impaired wound healing is accompanied by persistent high expression of Ang-2 and low expression of VEGF in the tissue (30). This is in a concordance with the theory that Ang-2 is involved in vessel regression in case of low expression of endothelial mitogenes (i.e. VEGF) in the tissue (31). In diabetes mellitus, chronic hyperglycemia causes an accelerated formation of advanced glycation end products (AGE) and mitochondrial overproduction of reactive oxygen species (ROS). The resulting toxic and oxidative stress in vascular endothelium promotes micro- and macro-vascular complications (32). Amongst multiple pathological changes in gene expression, AGE and ROS lead to the up-regulation of Ang-2 mRNA expression (33), which promotes vascular permeability, destabilization and sprouting (34). The explanation of lower plasma Ang-2 level cannot be explain by this study and need molecular evaluation of Ang-2 receptors in retina and kidney of T1DM patients and ratio between Ang-1 and Ang-2 circulating levels.

Table 1. Demographic characteristics of patients and controls.

Parameters	**Control** (n=40)	**Type 1 diabetes mellitus** (n=50)	**Significance**
Age (years)	10.50±2.06 (6.00-13.00)	11.04±2.65 (6.00-16.50)	0.289
Gender			
Male	20 (50.00%)	24 (48.0%)	
Female	20 (50.00%)	26 (52.00%)	0.509
Weight (kg)	26.88±5.38 (19.00-38.00)	30.74±9.44 (8.50-52.00)	**0.024**
Height (m^2)	1.27±0.12 (1.15-1.46)	1.32±0.14 (1.10-1.56)	**0.040**
Body mass index (kg/m^2)	16.94±1.82 (14.37-19.70)	17.30±3.37 (11.52-23.80)	0.543

Data are expressed as mean ± SD (minimum-maximum) or number (percentage)
P: significance versus control.

Table 2. Clinical characteristics of patients.

Parameters	**Type 1 diabetes mellitus** (n=50)
Positive family history	26 (52.00%)
Onset (years)	9.38±2.98 (1.00-14.00)
Duration (years)	1.71±2.46 (0.00-10.00)
Glycemic uncontrolled	33 (66.00%)
Duration	
Short duration (< 5 year)	40 (80.00%)
Long duration (≥5 year)	10 (20.00%)
Symptoms and signs	
Polyuria	41 (82.00%)
Polydipsia	41 (82.00%)
Abdominal pain	35 (70.00%)
Diabetic ketoacidosis	27 (54.00%)
Vomiting	16 (32.00%)
Septicemia	4 (8.00%)
Gingivitis	3 (6.00%)
Valvovaginitis	2 (4.00%)
Urinary tract infection	1 (2.00%)
Hospital stay (days)	9.44±2.69 (3.00-15.00)

Data are expressed as mean ±SD (minimum-maximum) or number (percentage)

Table 3. Measured metabolic and angiogenic parameters of patients and control.

Parameters	**Control** (n=40)	**Type 1 diabetes mellitus** (n=50)	**Significance**
HbA1c (%)	5.50±0.55 (4.60-6.30)	9.70±2.69 (5.00-13.00)	***P* <0.0001**
Glucose (mg/dl)	94.20±14.98 (68.00-119.00)	450.50±102.69 (195.00-600.00)	***P* <0.0001**
Insulin (ulU/ml)	5.43±2.81 (3.10-10.88)	24.53±19.82 (2.04-57.99)	***P* <0.0001**
VEGF (pg/ml)	72.27±13.09 (57.74-88.77)	172.25±36.94 (111.61-266.85)	***P* <0.0001**
Angiopoietin-2 (ng/ml)	8.01±1.92 (3.33-10.89)	5.53±4.18(0.20-21.49)	***P* <0.001**
Adrenomedullin (ng/ml)	1.98±1.43 (0.14-3.85)	1.69±1.52 (0.10-7.86)	*P* <0.351

Data are expressed as mean± SD (minimum-maximum); HbA1c: glycosylated hemoglobin; VEGF: vascular endothelial growth factor; *P*: significance versus control.

Table 4. Measured metabolic and angiogenic parameters of subgroups type 1 diabetes mellitus (T1DM) patients and control.

Parameters	**Control** (n=40)	**T!DM with short duration** (n=40)	**T!DM with long duration** (n=10)
HbA1c (%)	5.50±0.55 (4.60-6.30)	9.43±2.81(5.00-13.00)	10.80±1.87 (7.00-13.00)
		***P* <0.0001**	***P* <0.0001** ***P* <0.057
Glucose (mg/dl)	94.20±14.98 (68.00-119.00)	438.95±107.13(195.00-600.00)	496.70±68.69 (369.00-600.00)
		***P* <0.0001**	***P* <0.0001** ****P* <0.034**
Insulin (ulU/ml)	5.43±2.81 (3.10-10.88)	24.05±19.88 (2.04-57.99)	26.45±20.51 (6.01-51.86)
		***P* <0.0001**	***P* <0.0001** ***P* <0.651
VEGF (pg/ml)	72.27±13.09 (57.74-88.77)	169.35±35.29 (111.81-249.72)	183.84±42.96 (116.34-266.85)
		***P* <0.0001**	***P* <0.0001** ***P* <0.157
Angiopoietin-2 (ng/ml)	8.01±1.92 (3.33-10.89)	5.69±4.52(0.20-21.49)	4.90±2.47(1.84-9.77)
		***P* <0.003**	***P* <0.011** ***P* <0.511
Adrenomedullin (ng/ml)	1.98±1.42 (0.14-3.85)	1.76±1.65 (0.10-7.86)	1.39±0.91 (0.14-3.43)
		P <0.510	*P* <0.261 ***P* <0.477**

Data are expressed as mean± SD (minimum-maximum); HbA1c: glycosylated hemoglobin; VEGF: vascular endothelial growth factor; **P*: significance versus control; **P: significance versus T!DM with short duration.

Table 5. Correlation (r, P) between measured parameters in diabetic patients.

Parameters	**HbA1c**	**VEGF**	**Insulin**	**Glucose**	**Angiopoietin-2**
VEGF	**0.266(0.031)**				
Insulin	-0.093 (0.260)	-0.154 (0.143)			
Glucose	**0.670(0.0001)**	**0.258 (0.035)**	-0.009 (0.476)		
Angiopoietin-2	-0.067 (0.323)	0.227 (0.057)	-0.201 (0.081)	-0.032 (0.412)	
Adrenomedullin	-0.042 (0.386)	0.106 (0.233)	**-0.253 (0.038)**	-0.055 (0.352)	**0.434(0.001)**

VEGF: vascular endothelial growth factor; HbA1c: glycosylated hemoglobin.

In experimental animal models of diabetes Ang-2 is upregulated resulting in excess expression of Ang-2 over Ang-1. Ang-1, Ang-2 and Tie-2 expressions are dysregulated in streptozotocin diabetic rats when compared with control animals. Specifically, in the early phase of the disease, a parallel upregulation of both Ang-1 and Ang-2 is observed, and, as the disease progresses, a progressive down regulation of Ang-1 expression occurs. Conversely, Ang-2 levels remain elevated resulting in a decreased Ang-1/Ang-2 ratio (35) and secondary Tie-2 receptor dysregulation. In experimental animal models of diabetes Tie-2 expression is upregulated at the glomerular level (36). A cause–effect relationship between excess Ang-2 and albuminuria has been

proposed in animals with inducible podocyte-specific overexpression of Ang-2 (37).

VEGF is the factor essential for virtually all aspects of endothelial function such as proliferation, migration, permeability and nitric oxide production and release and rendering the endothelium anti-apoptotic. In this study, we have confirmed previous observations of increased plasma VEGF levels in diabetic patients (28,38). Also, VEGF was significantly higher in T1DM with long and short duration versus healthy controls. A publication from other authors (8, 9, 10, and 39) indicates that VEGF can be an important factor influencing complications related to microangiopathy in patients with T1DM. In this study, a positive correlation was found between VEGF and blood glucose in T1DM patients. It is known that persistent hyperglycaemia, leading to the elevation of HbA1c level, may lead to the production and accumulation of AGE. The formation of AGE promotes production of pro-inflammatory cytokines, which may further initiate the increase of VEGF level. In diabetes, monocytes display a reduced response to VEGF, which has been described as ''VEGF resistance''. This invalid monocyte response is a result of their preactivation with AGE interfering with physiological stimulation pathway (40). As a result, VEGF in diabetic patients exerts a strong proinflammatory effect contributing to leukocyte stasis within the vascular leakage and blood-retinal barrier breakdown (41). Pediatricians are interested in receiving therapeutic tools for young patients with diabetes, aiming at limited complications on the microvascular level (42). Attempted to use the methods of gene therapy using adeno-VEGF in non-obese mice revealed that inhibition of VEGF expression may contribute to the inhibition of late diabetic complications or to their complete remission. In this study, no significant correlation was found between Ang-2 and VEGF. Significant associations between VEGF and Ang-2 levels have been noted in earlier studies in patients with various tumors (43), diabetes mellitus (29), and asthma (44).

In the present study, T1DM patients without vascular complications showed insignificant change in plasma AM levels compared to controls. Also, insignificant changes were found in T1DM with long and short duration compared to healthy controls. In contrary to our results, others studies reported significant increase in plasma AM in hyperglycemic patients compared with normal volunteers (45,46). Meanwhile another study (47) found that when patients with nephropathy were excluded, plasma levels of AM were not significantly different in old diabetic patients and healthy individuals. Other authors reported that diabetic patients with microvascular complications displayed higher AM levels compared to those without (18, 19, and 48). Higher levels of AM and cAMP has been reported in patients with renal insufficiency but normal in micro-albuminuric patients (18). Adrenomedullin could exert a wide range of vascular actions (mostly protective). These include endothelium-dependent and -independent vasodilatation, antioxidative stress, stimulation of endothelial nitric oxide production, antiproliferation of vascular smooth muscle cell, and adventitial fibroblast (49).T1DM patients included in this study were without vascular complications. It had been reported that the increased of AM levels was in patients with longer duration of diabetes and it correlates with poor glucose metabolic control in T2DM (18, 50), suggesting that the elevation of AM levels is a late phenomenon due to endothelial dysfunction. The normal levels of patients without complications argue against a primary role of adrenomedullin in the initial generalized vasodilatation induced by diabetes, although a local paracrine effect cannot be excluded.

In this study, we found a positive significant correlation between AM and Ang-2 among diabetics which can be explained by that these 2 indices are important in angiogenesis. Also, a significant negative correlation was found between AM and insulin. Others (17) reported that AM inhibits insulin secretion both in vitro (isolated rat islets) and in vivo (oral glucose tolerance test in rats) in a dose-dependent manner. In accordance to other authors (18,51), we found that plasma AM levels did not correlate with plasma glucose, or HbA1c levels, suggesting that metabolic control, as assessed by these parameters, did not influence adrenomedullin levels.

Conclusions

The increase in plasma VEGF level in type 1 diabetes mellitus children and adolescents without vascular complications and its association with metabolic control which is known to be high risk factor for microvascular complication is an interesting and may declare its role in the pathogenesis of diabetic microangiopathy since childhood. Also, the lower plasma level of Ang-2 in these patients need worth studying with a larger patient material to confirm our finding. Our analysis of VEGF, Ang-2 and adrenomedullin levels in T1DM pediatric patients without vascular complications forms a basis for future analyses of this factor in patients being treated with antiangiogenic regimen including inhibitors of VEGF.

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