

Proangiogenic factors in obese patients with type 1 diabetes

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Abstract

Obesity is regarded as one of the main factors predisposing for type 2 diabetes. Recently many studies have shown that obesity and insulin resistance take part in pathogenesis of diabetes type 1 in children and adolescents. Angiogenin, TNF- α and VEGF are considered inducers of neovascularization. The levels of these cytokines may increase during the course of obesity and this may accelerate the progression of microvascular complications.

Since there are no data concerning the influence of obesity on the clinical status of patients with an established diabetes, we aimed to investigate the relationship between the level of three cytokines (angiogenin, VEGF, TNF- α) and body weight in diabetic children with reference to their clinical status and complications. The study was carried on 44 children with long-standing diabetes mellitus type 1 and on 30 healthy children as a control group. All the children had their daily urine albumin secretion, HbA_{1c}, C-peptide measured; 24 hrs blood pressure monitoring and ophthalmologic examination has been done. Body mass index values were compared to the appropriate centile charts. Serum level of vascular endothelial growth factor (VEGF), angiogenin and TNF- α (Tumor Necrosis Factor alpha) was measured by ELISA method.

Overweight patients with type 1 diabetes had significantly higher HbA_{1c}, total serum cholesterol, LDL cholesterol, tryglicerides, CRP and albumin levels than their healthy counterparts. Instead, these patients had lower HDL cholesterol level and higher systolic and diastolic blood pressure. In addition, the study group had higher VEGF level, higher TNF- α level and higher angiogenin level compared to normal-weight individuals and control group. Interestingly, obese diabetic type 1 patients more often suffered from the late diabetic complications (41% vs. 4%).

Concluding, elevated levels of angiogenin, VEGF and TNF- α may constitute disadvantageous factors in development of diabetic complications. Analyzed cytokines have negative effect on the course of the disease, and coexisting overweight accounts for the secondary risk factor for vascular complications. Thus, it is of great importance to monitor body-weight and to prevent overweight in patients with type 1 diabetes.

Key words: overweight, diabetes type 1, children, adolescents, VEGF, angiogenin, TNF- α .

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Introduction

Obesity is considered one of main factors predisposing to type 2 diabetes [1]. Recently many studies have shown that obesity and insulin resistance take part in pathogenesis of diabetes type 1 in children and adolescents [2]. Till now most of the researchers linked insulin resistance in diabetic type 1 patients with their clinical status, the risk of complications and the prognosis [3-9].

Accelerator hypothesis published in 2001 describes the association between increased body mass and type 1 diabetes development [2]. It proposes a model for diabetes pathogenesis whereby excess body weight contributes to insulin resistance resulting in beta-cell apoptosis [2]. So, body weight may become an additional environmental factor that accounts for incidence of diabetes type 1. According to Kilbrige et al. DM1 onset is associated with body weight [2]. It negatively

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correlates with BMI which may be evidenced by the fact that insulin dependent diabetes is recognized earlier in life in girls than boys. As it is thought, girls have genetically determined higher BMI resulting in higher insulin resistance [2]. Some researchers indicate that destruction of pancreatic islets in the individuals with insulin resistance resulting from glucotoxicity may be similar to autoimmune damage [2, 6]. The rate of these two processes is associated with genetic predisposition.

In children that are islet autoantibody-positive, but don't demonstrate any clinical features of the disease, the weight gain may accelerate an overt diabetes type 1 [2].

The existence of moderate inflammatory state is one of the features of obesity, as well as diabetes alone [10-15]. The markers of this state include cytokines TNF, IL-6, VEGF. Even though, there is no data concerning this subject, coexistence of these processes is assumed to force the inflammatory state, which may negatively influence the course of the disease. Angiogenin, TNF- α and VEGF are considered inducers of neovascularization [16-18]. The cytokine levels may increase in obesity accelerating the progression of microvascular complications [19-21].

Since there are no data concerning the influence of obesity on clinical status of patients with an established diabetes, we aimed to investigate the relationship between the level of three cytokines (angiogenin, VEGF, TNF- α) and body weight in diabetic children with reference to their clinical status and complications.

Material and Methods

The study was carried on 44 children of both genders with long-standing diabetes mellitus type 1 from the Department of Paediatrics, Haematology, Oncology and Endocrinology of the Medical University of Gdańsk and on 30 healthy children as a control group. Type 1 DM, was defined in accordance with the criteria of the American Diabetes Association (ADA, 2003). All patients with T1DM required insulin treatment (0.87 ± 0.24 U/kg of the body weight).

All the children had their daily urine albumin secretion, HbA1c, C-peptide measured; 24 hrs blood pressure monitoring and ophthalmologic examination has been performed.

Laboratory examinations

HbA1c was measured by an immunoturbidometric method using Unimate 3 set (Hoffmann-La Roche AG, Germany) with a normal range of values 3.0-6.0%. Fasting glucose was measured by enzymatic test (Roche Diagnostics GmbH, Germany). Level of CRP was measured with immunochemical system (Beckman Instr. Inc., Ireland). Level of the C-peptide was below 0.5 ng/ml in all children with type 1 DM. The urinary albumin excretion was expressed as the average of the three 24-hour collections obtained during 6 months prior to the enrolment into the study. Micro-

albuminuria cases were identified when at least in two out of three urine samples albumin excretion was between 30-299 mg/24 hours. The urinary albumin excretion is measured by immunoturbidometric assay using Tina-quant (Boehringer Mannheim GmbH, Germany). Serum level of creatinine was measured using CREA assay system (Boehringer Mannheim GmbH, Germany). Ophthalmologic investigation was performed in all children with T1DM. This included visual acuity tests, intraocular pressure, and anterior segment estimation by slit lamp (Topcon SL-82 Japan). The fundus examination was done following installation of 1% Tropicamid to obtain sufficient mydriasis. The examination was performed using the +90D lens (Ocular Instruments USA). A digital camera (Topcon Imagent 2000 Japan) was used to perform the fluorescein angiography. Analysis of the eye fundus pictures was based on The International Diabetic Retinopathy Division (ETDRS, 1991). The systolic and diastolic blood pressures were measured using automatic 24-hour ambulatory blood pressure monitoring (ABPM). Body mass index values were compared to the appropriate centile charts [22-24].

The study was conducted on three groups: patients with long-standing T1DM with coexisting overweight (n = 22); diabetic patients with normal body weight (n = 22) and a healthy control group.

Serum level of vascular endothelial growth factor (VEGF), angiogenin and TNF- α (Tumor Necrosis Factor alpha) was measured by immunoenzyme ELISA method (Quantikine Human by R&D System, Minneapolis, Minn., USA) according to manufacturer protocol. This study was approved by The Ethics Committee of The Medical University of Gdańsk NKEBN/610/2003/2004 and the investigation was carried out in accordance with the principles of the Declaration of Helsinki as revised in 1996.

Statistical analysis

The results were analyzed using The Statistical Version 7.0 program (StatSoft Polska). The Shapiro-Wilk test was used to evaluate normality of variables. The differences between the groups were calculated with Student t or the nonparametric U-Mann-Whitney tests.

The results of Student t test were presented as arithmetic means \pm SD. The results of the U-Mann-Whitney test were presented as median (min-max). In all analyses a two-tailed significance level <0.05 was regarded as statistically significant.

Results

As shown in Table 1, overweight patients with type 1 diabetes had significantly higher HbA1c, total serum cholesterol, LDL cholesterol, triglycerides, CRP and albumin levels than their healthy counterparts. Instead, these patients had lower HDL cholesterol level and higher systolic and diastolic blood pressure. In addition, the this study group had higher VEGF level (Fig. 2), higher TNF- α level (Fig. 3)

Table 1. Clinical characteristics of DM1 patients

	Patients with DM1 and overweight	Patients with DM1	Control group	p
Numer (n)	22	22	30	–
Gender (years)	13.5 ± 5.0	13.3 ± 5.3	14 ± 4	0.2 * 0.04**
Duration of disease (years)	4.4 ± 3.2	6.5 ± 1.8	–	0.002* –
BMI (kg/m ²)	25.5 ± 1.1	18.4 ± 3.2	16.6 ± 2.4	0.00001* 0.00000000**
Total cholesterol (mg/dl)	180 ± 43	166 ± 32	156.4 ± 19.1	0.7* 0.00000000**
HDL Cholesterol (mg/dl)	57 ± 15	64 ± 16	81.1 ± 21.8	0.03* 0.00000000**
LDL Cholesterol (mg/dl)	94 ± 33	87 ± 22	45 ± 14	0.5* 0.00000000**
Triglycerides (mg/dl)	95 ± 45	74 ± 38	89.7 ± 30.8	0.02* 0.00000000**
HbA1c (%)	9.5 ± 1.5	7.6 ± 1.1	4.2 ± 0.3	0.001* 0.00000000**
CRP (mg/dl)	1.7 ± 1.0	1.3 ± 0.7	0.7 ± 0.5	0.04* 0.00000000**
Albumin excretion (mg/24h)	43.0 ± 18.3	10.9 ± 8.5	4.2 ± 0.3	0.005* 0.00000000**
Systolic blood pressure (mmHg)	120 ± 9	111 ± 12	110 ± 8	0.02* 0.00000000**
Diastolic blood pressure (mmHg)	76 ± 8	72 ± 7	65 ± 7	0.24* 0.001**
Complications (%)	9 (41%)	1 (4%)	–	

* Patients with DM1 and overweight vs. patients with DM1; ** patients with DM1 vs. Control Group.

and higher angiogenin level (Fig. 1) compared to normal-weight individuals with DM and control group. Interestingly, obese diabetic type 1 patients more often suffered from the late diabetic complications (41% vs. 4%).

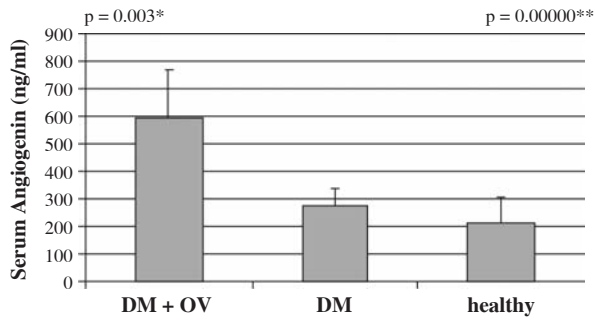
Correlations were found between BMI as well as CRP values and cytokine levels (Tables 2 and 3) which, however, lacked statistical significance.

Discussion

A group of 44 young patients with DM1 was examined. All children were under permanent care of The Diabetic Department, Clinic of Pediatrics, Hematology, Oncology and Endocrinology Medical University of Gdańsk. The control group consisted of 30 healthy children aged 14 ± 5.

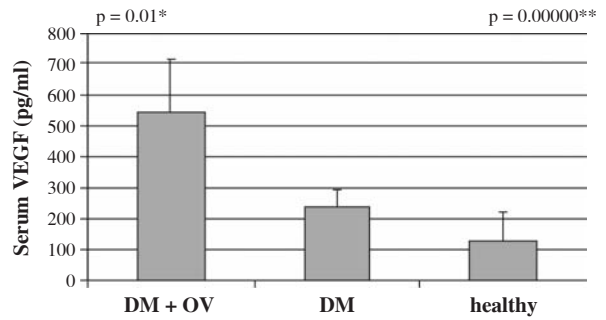
As it was previously shown the obesity may be low-grade systemic inflammatory disease [13, 15, 25] characterised by upregulation of proinflammatory cytokines, the elevated levels of which may accelerate the development of vascular

complications [11, 12, 15]. Low grade inflammatory conditions underlie diabetes mellitus course [14]. Microangiopathy develops as a consequence of a prolonged process associated with non-enzymatic protein glycation [26]. Glycated substances activate leucocytes, monocytes and macrophages. Cellular products of immunocompetent cells increase the permeability of endothelial cells and change their metabolism [15, 26]. Through the MAPK and NF-κB pathways endothelial cells start to produce cytokines by themselves potentiating the inflammatory conditions in small vessels, for example in retina and kidney. At the same time these cytokines attract new activated blood cells which infiltrate walls of the vessels [14, 15, 26]. Inflammatory cytokines recruit different growth factors including those stimulating angiogenesis [14]. The excessive body weight in our diabetic patients was associated with worse lipid profile, lack of metabolic compensation as well as higher blood pressure. These results were consistent with those obtained by other groups [8, 24, 25, 27-30].



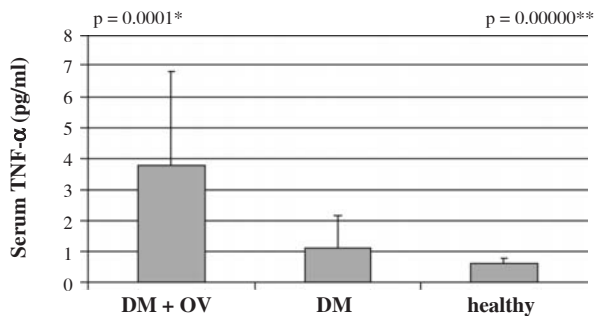
DM + OV – children with diabetes mellitus with coexisting overweight; DM – children with diabetes mellitus; healthy – healthy control group. * DM + OV vs. DM. ** DM vs. healthy

Fig. 1. The level of angiogenin in patients with diabetes



DM + OV – children with diabetes mellitus with coexisting overweight; DM – children with diabetes mellitus; healthy – healthy control group. * DM + OV vs. DM. ** DM vs. healthy

Fig. 2. The level of VEGF in patients with diabetes



DM + OV – children with diabetes mellitus with coexisting overweight; DM – children with diabetes mellitus; healthy – healthy control group. * DM + OV vs. DM. ** DM vs. healthy

Fig. 3. The level of TNF-α in patients with diabetes

Table 2. Correlation between BMI and cytokine levels

Comparison	r	p
BMI vs. Angiogenin	0.25	0.55
BMI vs. VEGF	0.36	0.37
BMI vs. TN-α	0.64	0.08

Table 3. Correlation between CRP and cytokine levels

Comparison	r	p
CRP vs. Angiogenin	0.54	0.16
CRP vs. VEGF	0.27	0.51
CRP vs. TNF-α	0.42	0.3

Metabolically active adipose tissue is a good source of cytokines, that have an effect on endothelial cells and influence the process of angiogenesis. It was earlier observed that the nature of angiogenesis may be modified in obese individuals [10]. Thus the obesity may accelerate tumor growth [10]. These observations suggest that obesity may be associated with the increase in expression of proangiogenic factors, which may contribute to pathogenesis of obesity or simple may be a consequence of an excessive body weight. Irrespective of the cause of their high levels, these cytokines contribute to the development of diabetic complications [14]. Thus we aimed to investigate whether the level of these cytokines in the group of overweight diabetic patients is elevated and whether it is associated with vascular complications.

Our studies indicate that overweight patients with DM1 have higher levels of all angiogenic cytokines in comparison with control non-obese DM1 group. Interestingly, we observed that in this group up to 40% of patients had

diabetic complications, while in the non obese diabetic group only 4 % of patients suffered from complications.

Angiogenin is a small protein detectable in sick as well as healthy individuals [18, 20, 31-33]. Even though there is no known receptor for this cytokine, it's reactivity contributes to alterations in angiogenesis, which in turn may have impact on sudden development of diabetic vascular complications. Previously, it was shown that the level of angiogenin is lower in type 2 diabetic patients [32]. In our study population the level of this cytokine was significantly higher in type 1 diabetic patients in comparison to their healthy counterparts. In addition the level of angiogenin was far higher in overweight diabetic type 1 individuals which is consistent with study by Guo-Fu Hu et al. [20].

The role of VEGF in pathogenesis of diabetes seems to be well known. VEGF contributes to development of diabetic retinopathy [17]. We have showed it's higher level in our previous studies [17, 33, 34], and this is consistent with results of other researchers [36, 37]. However, there

are no data concerning the association of the serum level of VEGF and body mass. It is only known that together with other cytokines, VEGF has impact on angiogenesis in adipose tissue [19] which is proved by higher level of VEGF in overweight DM1 patients in our study group. The adipose tissue angiogenesis may be also mediated by TNF- α which is produced by adipocytes [10, 21]. TNF- α is also strictly associated with diabetic complications [34] and involved in insulin resistance as shown on adipocytes of obese mice [38]. In our studies the TNF- α level was higher in DM1 diabetic patients, which was also reported in other papers [21, 34]. Moreover, similarly to angiogenin and VEGF levels, the level of TNF- α was higher in overweight DM1 patients in comparison with normal-weight DM1 individuals.

We were not able to find statistically significant correlations between BMI, CRP values and concentration of cytokines. This fact was most probably due to small size of the examined group. Moreover, our DM1 patients were different with respect to age and sex. Meanwhile, maturation is associated with an increase in BMI level.

To conclude, the elevated levels of angiogenin, VEGF and TNF- α may constitute disadvantageous factors in development of diabetic complications. Analyzed cytokines have negative effect on the course of the disease, and coexisting overweight accounts for the secondary risk factor for vascular complications. Thus, it is of great importance to monitor body-weight and to prevent overweight in patients with type 1 diabetes.

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