Relationship between serum levels of tumor necrosis factor-alpha and interleukin-6 in diabetes mellitus type 1 children

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Abstract

It has been documented that the tumor necrosis factor α (TNF-α) serum level correlates with the severity of diabetic retinopathy. No data, however, is available on its presence in an earlier phase of this complication. The aim of our paper was to find out whether a detection of TNF-α serum level in children with Type 1 diabetes mellitus (DM) may be a useful marker of diabetic retinopathy (DR). Eighty seven children with type 1 DM were categorized into two groups: those with diabetic retinopathy and without retinopathy. The levels of TNF-α and interleukin-6 (IL-6) were measured in the serum. The children with DR were older and demonstrated a significantly longer duration of the disease in addition to a higher HbA_1c values, albumin excretion rate, C reactive protein and HDL-cholesterol levels as well as the systolic blood pressure than those without retinopathy. The logistic regression revealed that the risk of DR was strongly dependent on TNF-α {(OR 4.01; 95%CI 2.01-7.96) p=0.0001}. Other inflammatory mediators were also indicators of retinopathy, though with less power: CRP {(OR 2.59; 95%CI 1.56-4.30) p=0.0001} and IL-6 {(OR 1.81; 95%CI 1.26--2.59) p=0.001}. An elevated level of TNF-α serum may be a marker of diabetic retinopathy in DM children. Our study implies that DM children who did not progress into retinopathy yet but have detectable serum level of TNF-α should be provided with careful ophthalmologic control.

Key words: *TNF-α, IL-6, diabetes mellitus, retinopathy, children.*

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Introduction

In the course of the diabetes the main clinical problem to be faced is the development of micro- and macro-angiopathy. Nephropathy, retinopathy and arterial hypertension are present in children already 5 years from the disease onset [1]. Regardless numerous studies carried out worldwide, daily urine albumin secretion remains to be standard marker of nephropathy development, while ophthalmologic examination stands for diagnosis of retinopathy [2, 3]. Therefore in the recent years we have decided to focus our research on the search for markers of diabetic damage to kidneys and eye apparatus already at the early phase of the disease, when the widely used parameters of function of these organs remain still within the range of norm. Presently, there is no satisfactory pharmacologic therapy and laser therapy remains to be the leading treatment option in management of DM1 retinopathy in children [4, 5]. Yet still the therapy is aimed on limiting the damage and not on preventing it. Therefore early detection of patient's tendency for retinopathy development, at the stage when still no damage is visible at the level of the eye fundus, remains of tremendous importance especially in DM1 children and young adults.

Our group in formerly published studies has shown that TNF-α appears, from the group of studied parameters, to be the significant marker of nephropathy preceding to the mi-

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croalbuminuria [6, 7]. Interleukin-6 has not been analyzed of DM1 retinopathy children.

Therefore, the aim of our paper was to compare the serum concentration of TNF-α in children with several years duration of diabetes mellitus complicated and non-complicated with DR. These values were analysed in terms of the clinical condition of the patients. Moreover, the presence of retinopathy was related to the serum concentration of IL-6 the too proinflammatory cytokine, known to be regulated by TNF-α [8].

Material and Methods

Eighty seven children (48 girls and 39 boys, age 13.6±3.74 years) with type 1 DM were recruited from The Outpatient Diabetic Department at The Medical University of Gdańsk. Type 1 DM, was defined in accordance with the criteria of the American Diabetes Association [9]. The analysis of the eye fundus pictures was based on The International Diabetic Retinopathy Division: 1) non-proliferative retinopathy with or without maculopathy, 2) pre-proliferative retinopathy, 3) proliferative retinopathy [2]. According to the ophthalmological examination the diabetic children were devided into 2 groups: with diabetic retinopathy (Group A=17 children) and without retinopathy (Group B=70 children). In 17 cases, typical symptoms of non-proliferative diabetic retinopathy were proven. Within the retinopathy group 10 children had concomitant microalbuminuria and six were affected with arterial hypertension. The DM patients without retinopathy were all free from microalbuminuria and arterial hypertension. A group of 35 healthy children (18 girls and 17 boys, age 13.08±3.6 years) volunteered as a control group. They were apparently healthy as based on their medical examination. The chlidren with symptoms of infection or systemic somatic illnesses other than diabetes mellitus were excluded from the study. Informed consent was obtained from each subject (or from his/her parents) participating in the study. This study was approved by The Ethics Committee of The Medical University of Gdańsk NKEBN/610/2003/2004.

Methods

HbA_1c 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). Levels of total cholesterol, HDL-cholesterol and triglycerides were measured on the enzymatic kits : Comray Chol, Comray HDL-Direct and Comray-TG (P.Z. Comray, Poland). Level of CRP was measured with immunochemical system (Beckman Instr. Inc, Ireland). Level of C-peptide was below 0.5 ng/ml in all children with type 1 DM. The systolic and diastolic blood pressure were measured using automatic 24 hour ambulatory blood pressure monitoring (ABPM) and all the average values of the blood pressure were expressed in the centyle charts [10]. The urinary albumin excretion was expressed as the average of the three 24h collections obtained during 6 months prior to the enrloment into the study. Classified as microalbuminuria were the cases when in at least in two out of three urine samples albumin excretion was between 30-299 mg/24hrs. The urinary albumin excretion 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). All patients required insulin treatment (0.87±0.24 IU/kg/mass of body). In all children with type 1 DM the ophthalmological investigation was performed. This included visual acuity tests, intraocular preasure, anterior segment estimation done by slit lamp (TOPCON SL-82 Japan). The fundus examination was done after instalation of 1% Tropicamid to obtain sufficient mydriasis. The examination was performed by using the +90D lens (Ocular Instruments USA). Digital camera (Topcon Imaginet 2000 Japan) was used to perform the fluorescein angiography.

Blood collection

Blood samples were collected between 8 and 9 after an overnight fast. The sera were separated from the venous blood within 30 min and kept frozen at –80°C up to three months prior to analysis. All determinations were done on the same blood sample.

Detection in serum of TNF-α and IL-6 levels

Serum levels of cytokines; TNF-**α** and IL-6 were measured by immunoenzymatic ELISA method (Quantikine High Sensitivity Human by R&D Systems, Minneapolis, Minn., USA) according to manufacturer protocol. Minimum detectable concentrations were determined by the manufacturer as 0.12 pg/ml and 0.03 pg/ml respectively. Intra-assay (2.6 for TNF-α; 1.6 for IL-6) and inter-assay (7.4 for TNF-α; 6.4 for IL-6) precisions performances of the assays were determined on 20 replicates from the quality control data of the laboratory.

Statistical analysis

The results were analysed using The Statistica, Version 7.0 program (StatSoft, Pl). The Shapiro-Wilk's test was used to evaluate normality of variables. The differences between the groups were calculated with T Student's or the non- parametric U Mann Whitney tests. A logistic forward regression analysis was used to assess the association between all clinical and inflammatory parameters and retinopathy with a P value <0.5 for entry. Risk for retinopathy was estimated by odds ratios (ORs) with 95% confidence intervals (CIs). The χ^2 test was applied for calculating differences between numbers of children with and without detectable cytokines in serum.

Results

Basic clinical parameters of DM and healthy children

The study was carried out on a group of 87 children diagnosed with diabetes mellitus (DM) type 1 and 35 healthy children. The diabetic children were characterised by significantly higher HbA_1c, CRP, serum creatinine and fasting blood glucose levels as well as albumin excretion rate than the healthy control group. The DM children had also higher systolic and diastolic blood pressures in relation to the healthy children. Triglycerides, total-cholesterol, HDL- and LDL--cholesterol levels were similar in the both groups (table 1).

Clinical parameters of children with (Group A) and without retinopathy (Group B)

The children with retinopathy were older and demonstrated a significantly longer duration of the disease in addition to higher HbA_1c, albumin excretion rate, C reactive protein, HDL-cholesterol levels as well as systolic blood pressure than those without retinopathy. There were no significant differences between both groups in the serum creatinine, glucose, triglicerydes, total cholesterol, LDL--cholesterol levels and diastolic blood pressure. Within the retinopathy group 10 patients had concomitant microalbuminuria and six were affected with arterial hypertension. The DM patients without retinopathy were all free from microalbuminuria and arterial hypertension (table 2).

Level of TNF and IL-6 in serum of with and without retinopathy children and control group

The level of TNF-α in the group with DR was significantly higher 2.3±1.0 pg/ml as compared without retinopathy children 0.7±0.5 pg/ml (p=0.00001) and in relation to the healthy group 0.0 pg/ml (p=0.00001) (table 3).

The level of IL-6 in the children with DR was significantly higher 3.8±1.7 pg/ml as compared without retinopathy children 1.6±0.9 pg/ml (p=0.0004) and in relation to the healthy control group 0.3±0.1pg/ml (p=0.01) (table 3).

The logistic regression revealed that the risk of retinopathy was dependent with declining power on: TNF-α

Table 1. Basic clinical parameters of DM and healthy children

Clinical parameters	DM children	Healthy group	p
number of children	87	35	
age (years)	16.0±2.5	15.5±2.2	0.3
duration of diabetes (years)	8.8±3.5	-	-
HbA_1c (%)	9.7±1.8	4.2±0.3	0.01*
CRP mg/l	2.32±0.99	0.3±0.04	0.0001*
albumin excretion rate (mg/24)	43.98±26.28	2.8±1.2	0.01*
creatinine in serum (μmol/l)	0.97±0.9	0.5±0.1	0.002*
fasting blood glucose (mmol/l)	12.6±8.6	4.93±0.69	0.008*
triglycerides (mmol/l)	1.46±0.75	1.02±0.35	0.5
total cholesterol(mmol/l)	4.48±1.00	4.01±0.49	0.2
HDL-cholesterol (mmol/l)	1.54±0.42	1.15±0.36	0.3
LDL-cholesterol (mmol/l)	2.6±0.91	2.08±0.56	0.4
systolic blood pressure (mm Hg)	125.5±11.4	110.0±10.0	0.001*
diastolic blood pressure (mm Hg)	75.0±10.0	68.0±8.0	0.03*

*Values are presented as means ± SD. *differences between diabetic children and healthy group. * – statistical significance (p<0.05).*

Table 2. Basic clinical parametrs of children with (Group A) and without retinopathy (Group B)

Parameters	Group A=17	Group B=70	Statistical significance
age (years)	15.72±3.54	13.08±3.6	p=0.02*
duration of diabetes (years)	8.4±3.7	4.6±2.8	p=0.0007*
HbA_1c (%)	9.7±1.9	8.0±1.66	p=0.01*
CRP mg/l	2.32±0.99	1.44±0.83	p=0.03*
albumin excretion rate (mg/24)	35.40±26.4	15.59±8.9	p=0.01*
creatinine in serum (μmol/l)	78.76±8.84	74.26±11.49	p=0.1
fasting blood glucose (mmol/l)	8.9±1.63	9.00±4.13	p=0.54
triglycerides (mmol/l)	1.0±0.42	0.92±0.87	p=0.1
total cholesterol (mmol/l)	4.78±1.07	4.40±1.01	p=0.4
HDL-cholesterol (mmol/l)	1.55±0.33	1.39±0.35	p=0.03*
LDL-cholesterol (mmol/l)	2.46±0.75	2.21±0.60	p=0.81
systolic blood pressure (mm Hg)	122.5±11.4	112.76±11.13	p=0.001*
diastolic blood pressure (mm Hg)	73.0±10.0	70.2±8.9	p=0.3

*Vales are presented as means ± SD. *differences between children with Group A and Group B. * – statistical significance (p<0.05).*

Table 3. Level of cytokines in serum of children with (Group A) and without retinopathy (Group B) and healthy control group

Cytokines	Group A=17	Group B=70	Healthy group	Statistical significance
TNF-α (pg/ml)	2.3±1.0	0.7±0.5	0.0	p=0.00001* p=0.000001**
IL-6 (pg/ml)	3.8±1.7	1.6±0.9	0.3±0.1	p=0.0004* p=0.01**

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{(OR 4.01; 95%CI 2.01-7.96) p=0.0001}, CRP {(OR 2.59; 95%CI 1.56-4.30) p=0.0001}, IL-6 {(OR 1.81; 95%CI 1.26-2.59) p=0.001} and albumin excretion rate {(OR 1.01; 95%CI 1.0-1.36) p=0.04}.

Discussion

We examined a group of 87 children with type 1 DM, including 17 children with recognised DR. The DR children were older and demonstrated a significantly longer duration of the disease in addition to a higher HbA_1c, albumin excretion rate, C reactive protein level, HDL-cholesterol levels as well as the systolic blood pressure than those without retinopathy.

The level of TNF-α appeared to be the most powerful independent marker of retinopathy as it increased 4 times the risk of DR in children. The serum concentrations of CRP and IL-6 levels and the albumin excretion rate were also significant but weaker risk factors. The concentration of serum TNF-α serum in the DR children by far exceeded that in the DM children without complications. This finding points to the significance of TNF-α and other proinflammatory mediators as the early diabetic retinopathy risk factors. Thus, TNF-α was placed at the top of inflammatory risk markers. This implies that DM children with the detectable serum TNF-α but without DR should be provided with careful ophthalmologic control.

TNF-α plays a significant role both in diabetes mellitus type 1 complicated and non-complicated. However, its role is not fully understood and the published results remain inconsistent. For instance, an Italian group that analysed TNF-α levels in the serum of children with newly diagnosed diabetes mellitus type 1 reported that the TNF-α level measured in the serum of the diabetic children was lower then that of the healthy group [11]. They suggested that an aberrant TNF-α synthesis may contribute to immune deregulation thus favouring the development of this autoimmune disease. Moreover, experiments in non-obese diabetic mice (NOD) indicate that the administration of exogenous TNF-α suppresses the development of diabetes mellitus in these known low producers of endogenous TNF-α [12-14]. Conversely, the results published by other groups are totally distinct. For example, Ng et al. [15] and Hui-Chen et al. [16] analysed a group of children of less than a 5-year duration of diabetes mellitus type 1 and with no late complications of the disease. They showed that the TNF-α serum levels did not differ from those of the healthy children.

The DR children, apart from an elevated TNF-α level, had also increased levels of such as IL-6. Interleukin 6 appeared to have achieved higher levels in the DR children compared with those without retinopathy and the control children. The DR children had also a higher median level of this cytokine. Some studies indicate that this pro-inflammatory cytokine, inducing the synthesis of acute phase proteins, initiates and supports an inflammatory process in the vascular wall, leading to its impairment [17, 18]. In harmony with this mechanism, we found that the DR children had a significantly higher concentration of CRP in relation to those without complications. The negative role of IL-6 is also associated with its increasing permeability effect on endothelial cells [19-21]. What is interesting, higher TNF-α and IL-6 levels have been found in the children with newly diagnosed type 1 DM as compared with the children suffering from long-lasting complicated diabetes [18]. These differences can be ascribed to the effect of a diabetes treatment and balancing the metabolic control.

It appeared that a significant inflammatory process underlies non-proliferative diabetic retinopathy, with TNF-α as the most important factor. Moreover, our results have documented that TNF-α serum is detectable in diabetic retinopathy children. Our study implies that DM children who did not progress into retinopathy yet but have detectable serum level of TNF-α require a pending careful ophthalmologic control.

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