Interleukin 18 and sICAM-1 serum levels in families with type 1 diabetes mellitus

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Abstract

It is well known that subjects with type 1 diabetes are at an increased risk of death from coronary heart disease in comparison to non-diabetic age-matched individuals because hyperglycaemia is believed to be a key risk factor for the development of micro- and macrovascular complications. On the other hand there is increasing evidence about the role of inflammatory mediators in the pathogenesis of atherosclerosis and the development of acute coronary syndromes. It has been recently suggested that IL-18 and sICAM-1 have a strong predictive value for cardiovascular diseases and deaths in patients with coronary artery disease and/or in apparently healthy men.

The aim of our study was to estimate the serum levels of IL-18 and sICAM-1 in subjects with type 1 diabetes and their relatives, who share HLA diabetic susceptibility genes (with or without pancreatic autoantibodies), but still without glucose level disturbances, as an evaluation of the possible role of genetic predisposition to the presence of IL-18 in diabetic families. The study was carried out in 35 type 1 diabetic subjects, their 101 healthy first-degree relatives: 36 siblings and 65 parents and the control group consisted of 31 healthy volunteers.

We have found increased IL-18 and sICAM-1 levels in subjects with type 1 diabetes and their first degree relatives, who share diabetic HLA haplotypes: DRB1*03/DRB1*04 or DRB1*03/*04/DQB1*02 independently of their autoimmune status. There was a strong positive correlation between IL-18 and sICAM-1 levels in diabetic subjects and their first degree relatives without glucose level disturbances.

To our knowledge this is the first study, which suggests that sICAM-1 elevations could be a result of IL-18 overproduction in type 1 diabetic subjects and their first degree relatives. Since in previous studies IL-18 and sICAM-1 were found to be predictors of death in subjects with CHD, one could speculate that high levels of IL-18 observed in subjects with genetic predisposition, but still with normal glucose levels, are an in addition to hyperglycaemia, pathogenic factors responsible for a higher risk of acute coronary events in subjects with diabetes type 1.

Key words: coronary heart disease, diabetes type 1, interleukin 18, soluble Intercellular Adhesion Molecule 1.

Introduction

It is well known that subjects with type 1 diabetes have a two to four times increased risk of death from coronary heart disease in comparison to non-diabetic age-matched individuals and hyperglycaemia is believed to be a key risk factor for the development of micro- and macrovascular complications [1]. However, in contrast to the beneficial influence on microvascular complications, the role of good glycaemic control in the prevention of cardiovascular deaths isn't still well documented in diabetic patients [2,3].

On the other hand there is increasing evidence about the role of inflammatory mediators in the pathogenesis of atherosclerosis and development of acute coronary syndromes [4-6].

Interleukin 18 (IL-18) is a pleiotropic pro-inflammatory cytokine involved in the regulation of innate and acquired immune responses, playing a key role in autoimmune, inflammatory and infectious diseases [7]. IL-18 strongly stimulates INF-γ production by T cells and NK cells and in synergy with IL-12 promotes the development of Th1 responses [7,8].

The possible role of IL-18 in the pathogenesis of cardiovas-

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Table 1. **Serum concentration of IL-18 and sICAM-1 in type 1 diabetic patients, their first degree relatives and healthy controls**

	Type 1 diabetes (n=35)	Siblings (n=36)	Parents (n=65)	Healthy controls (n=31)
IL-18 (pg/ml)	157.3±80.1**	170.3±66.9**	146.9±72.6*	115.1±30.4
sICAM-1 (ng/ml)	357.2±69.4###	311.2±75.1##	322.0±74.6#	257.3±46.7

Data are means ±SD, P values vs controls: ** p<0.01, * p<0.05, ### p<0.000001, ## p<0.00002, # p<0.00003

cular disease was previously suggested by Blankenberg et al., who have found IL-18 to be a strong predictor of cardiovascular death in stable and unstable angina [9]. An increased expression of IL-18 has been reported in human atherosclerotic plaque and proposed to be involved in the plaque destabilisation [10,11]. Increased plasma IL-18 concentrations also correlated with the severity of myocardial dysfunction in patients with acute coronary syndromes [12].

The soluble form of intercellular adhesion molecule 1 (ICAM-1) is another marker of inflammation recently found to be related with cardiovascular mortality and ischemic stroke independently from other traditional risk factors [13-15]. ICAM-1 plays a crucial role in leukocytes migration from circulation into inflamed tissues and the soluble form of ICAM-1 is supposed to be cleaved during this process [13-15]. High levels of circulating ICAM-1 are associated with the early stages of atherosclerosis development and their concentrations were shown to correlate with pro-inflammatory cytokines: TNF-α, IL-6 [16,17].

Moreover it was recently shown in *in vitro* studies that IL-18 in time- and concentration-dependent fashion up-regulates the expression of ICAM-1 in the monocytes population in human PBMC. On the other hand ICAM-1/LFA-1 interactions induced by IL-18 are important for the enhanced production of INF-γ, TNF-α, IL-12 [18,19].

The aim of our study was to estimate the serum levels of IL-18 in subjects with type 1 diabetes and their relatives sharing HLA diabetic susceptibility genes (with or without pancreatic autoantibodies), but as yet with no glucose level disturbances, as an evaluation of the possible role of genetic predisposition for the determination of IL-18 in diabetic families.

In addition we have investigated the relationship between IL-18 and sICAM-1 serum levels as their relationship has been suggested in *in vitro* studies and both markers were found to have a strong predictive value for cardiovascular diseases and deaths in the patients with coronary artery disease and/or in apparently healthy men [9,14,20].

Material and methods

The study was carried out in 35 type 1 diabetic subjects (mean age 15.5±7.6 yrs, F/M=11/24, with good metabolic control – HbA_1C<7%) and their 101 healthy first-degree relatives: 36 siblings (16.1±9.9, F/M=13/23) and 65 parents (40.9±9.5 yrs, F/M=33/32). Diagnosis of type 1 diabetes was made according to the criteria defined by WHO in 1985, the presence of ketosis, low body mass index and need for insulin therapy.

The control group consisted of 31 healthy volunteers from our staff families (mean age 16.4±3.1, F/M=13/18), who had no family history of type 1 diabetes and other autoimmune diseases.

The concentrations of IL-18 were measured by ELISA using two monoclonal antibodies against two different epitopes of human IL-18 (MBL, Ltd, Nagoya, Japan). The sensitivity of the assay is 12.5 pg/ml.

sICAM-1 levels in the serum were determined by solid phase ELISA assay (Parameter, R&D Systems, Abingdon, UK; the minimum detectable concentration was 0.35 ng/ml).

Antibodies against glutamic acid decarboxylase (GADA) and against tyrosine phosphatase (IA-2A) were measured by RIA (Euroimmun, GmbH, Lübeck, Germany). HbA_1C was quantified by high-performance liquid chromatography (Bio-Rad, München, Germany).

The genotyping of HLA alleles associated with type 1 diabetes mellitus in the Polish population: DRB1*03, DRB1*0401, DQB1*02 was performed using SSP-PCR method as described previously [21,22].

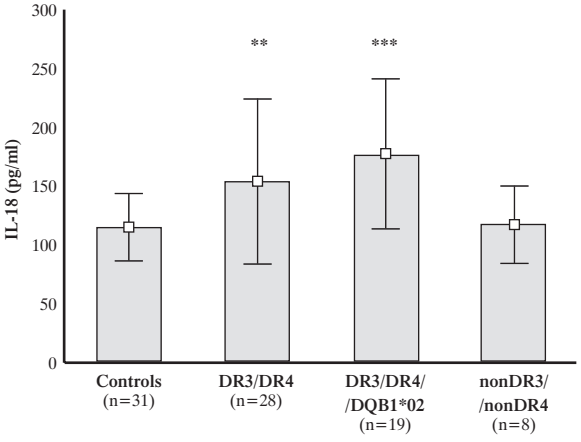
The results of IL-18 or sICAM-1 levels are presented as a mean ±SD. The statistical significance between the groups were evaluated by the Mann-Whitney U test, for regression analysis Spearman's correlation coefficient was used (Statistica 5.0, StatSoft, Tulsa, OK, USA) and the differences where considered significant at p<0.05.

Results

In subjects with type 1 diabetes fasting serum levels of IL-18 and sICAM-1 were increased in comparison to the healthy controls (*Tab. 1*) and the degree of these elevations were not dependent on the degree of glycaemic control. No correlation was observed between HbA_1C and IL-18 or sICAM-1 levels. We also didn't find any correlation between IL-18 levels and age or gender (data not shown).

Moreover our study has shown that IL-18 and sICAM-1 levels were higher in siblings and parents of diabetic subjects in comparison to the controls (*Tab. 1*). More detailed analysis revealed that increased IL-18 levels are observed in the first degree relatives, who share diabetic HLA haplotypes: DRB1*03/DRB1*04 or DRB1*03/*04/DQB1*02 (*Fig. 1*) independently of their autoimmune status (no difference in subjects with or without GADA, IA-2A autoantibodies). There was a strong positive correlation between IL-18 and sICAM-1 levels in diabetic subjects and their first degree relatives with no glucose level disturbances (r=0.4, p<0.0002) (*Fig. 2*).

Figure 1. **Serum concentration of IL-18 in association to HLA diabetic susceptibility alleles in siblings of type 1 diabetic patients**



** $p<0.03$ vs control group *** $p<0.006$ vs control group

Discussion

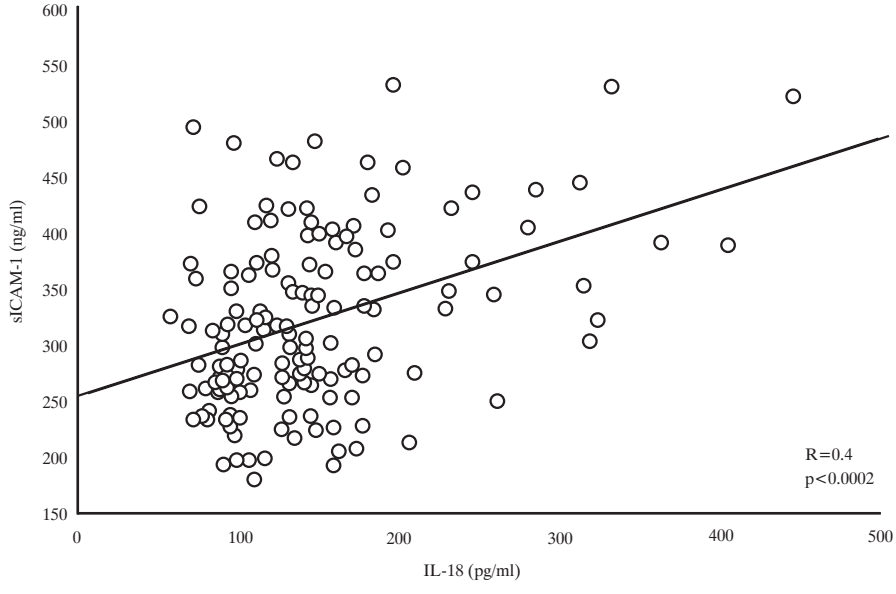
In our previous study we have shown an association between type 1 diabetes and G→C polymorphism at position -137 in the promoter of IL-18 gene [23]. These findings together with our present observations, that IL-18 levels are increased in type 1 diabetic patients and their healthy relatives, who share HLA diabetic susceptibility alleles, seems to suggest that IL-18 levels could be influenced by genetic predisposition in type 1 diabetic subjects and their relatives with alleles associated with the disease.

To our knowledge till now there has been only one paper published concerning the levels of IL-18 in type 1 diabetes [24]. Nicoletti et al have found higher levels of IL-18 in the pre-diabetic stage of type 1 diabetes, but in contrast to our results, not in the newly diagnosed subjects [24]. This difference could result from the methodological reasons, since their assay was able to measure detectable levels of IL-18 only in 3 out of 35 (8.6%) diabetic subjects. However It was recently reported that different ELISA assays can detect different subtypes of IL-18 in human serum [25]. In our assay two monoclonal antibodies were used against two different epitopes of human IL-18 (commercially available ELISA test, previously used in other published studies), IL-18 levels were detected in all the studied subjects and the mean levels of cytokine in the healthy controls were similar to the concentrations observed in other studies [9].

Moreover in the present study we found increased levels of sICAM-1 in type 1 diabetic patients and their first degree relatives and a strong positive correlation between IL-18 and sICAM-1 levels, which could suggest that high concentrations of sICAM-1 could be, at least partially, influenced by IL-18 overproduction in diabetic patients and their relatives.

In the present study we didn't observe any relationship between the both studied parameters and glycaemic control or immune status. No relation between HbA_1C or duration of disease and increased sICAM-1 in type 1 diabetes was previously reported by Fasching et al., who suggested that the high concentrations of sICAM-1 reflected endothelial cell stimulation and leukocyte activation [26]. Moreover Jude et al. observed that higher levels of ICAM-1 in the sera of patients with diabetes were of good predicture value of the development of macrovascular disease in diabetic patients independently of glycaemic control [14]. On the other hand Esposito et al. have shown that in subjects with IGT, as well as in healthy controls, acute hyperglycaemia increased IL-18 concentration during the clamp study [27]. In the light of their findings we believe that no correlation between HbA_1C and IL-18 levels in our study could

Figure 2. **Relationship between serum IL-18 and sICAM-1 levels in type 1 diabetic subjects and their first degree relatives**



result from good glycaemic control ($HbA_1C<7\%$) observed in the studied type 1 diabetic patients.

In summary, to our knowledge this is the first study which suggests that sICAM-1 elevations could be a result of IL-18 overproduction in type 1 diabetic subjects and their first degree relatives. Since IL-18 and sICAM-1 have been the predictors of death in subjects with CHD, one could speculate that higher levels of IL-18, observed in subjects with genetic predisposition to type 1 diabetes, but still with normal glucose levels, could be in addition to hyperglycaemia, considered a pathogenic factor responsible for a higher risk of acute coronary events in subjects with diabetes type 1 in comparison to non-diabetic age-matched individuals.

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