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Is cathelicidin a novel marker of diabetic microangiopathy in patients with type 1 diabetes?

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ABSTRACT

Aim: The aim was to evaluate the relationship between higher serum cathelicidin levels with the occurrence of chronic microangiopathic complications in patients with diabetes mellitus type 1 (DM1).

Methods: The study group consisted of 62 patients with DM1 (35 men), aged 30 (24–38) years and with duration of DM1 12 (9–17) years. Patients were divided into two groups depending on the level of cathelicidin, with cut-off point 24.5 ng/ml (median value for the whole group) and according to the presence or absence of any microangiopathy.

Results: The group with higher serum level of cathelicidin (n = 31) in comparison with patients with lower levels (n = 31) had higher serum level of total cholesterol [5.0(4.5–5.6) vs 4.5(3.9–5.0) mmol/l; p = 0.04], HDL cholesterol [1.9(1.5–2.1) vs 1.4(1.3–1.8) mmol/l; p = 0.009], LDL cholesterol [2.6(2.2–3.1) vs 2.3(1.9–2.8) mmol/l; p = 0.03] and higher TSH value [1.8(1.5–2.6) vs 1.4(0.9–2.1) mIU/L; p = 0.01]. Moreover, higher serum levels of cathelicidin were in women than men (58% vs 29%, p = 0.02) and in patients with vs without microangiopathy (45% vs 19%, p = 0.03). In the multiple regression model higher serum level of cathelicidin was related to the presence of microangiopathy, independently from sex, waist to hip ratio, serum total cholesterol level and TSH.

Conclusions: Patients with type 1 diabetes and presence of microangiopathy characterize higher level of serum cathelicidin. This observation may have important clinical implication and needs further investigations.

1. Introduction

Cathelicidin is an antimicrobial protein (AMP) that is significant for the human immune system. It affects directly broad spectrum of bacteria, viruses and fungi, as well as it presents immunomodulatory activity, affecting chemotactic function of leukocytes, monocytes and mastocytes [1]. Moreover, it stimulates angiogenesis and wound healing [2]. It appears that cathelicidin is a connection between innate and adaptive immune systems. Cathelicidin is secreted mainly by leukocytes, although AMP synthesis is also detected in the respiratory epithelium [3], skin cells and gastrointestinal tract [4]. Cathelicidin gene expression depends on many factors, such as vitamin D and pro-inflammatory cytokines (i.e. tumor necrosis factor alpha-TNF α). AMPs are essential in initiating inflammatory reaction, but also are responsible for ceasing it [5]. Abnormal secretion of cathelicidin appears to be connected with different diseases. Noticeably elevated levels of cathelicidin has recently been discovered in psoriasis, rosacea, rheumatoid arthritis and systemic lupus erythematosus [6]. Then, considerably low levels of AMP are connected with atopic dermatitis, Crohn's disease [7] and chronic skin ulcers.

Recent studies revealed that cathelicidin synthesis occurs also in adipocytes. The adipose tissue is not only a magazine for fat and energetic resources, but also is an active organ, that is an important part of the endocrine and immune system. It has been proved that preadipocytes and adipocytes secrete wide range of pro-inflammatory cytokines, hormones, growth factors and AMP affecting the inflammation, appetite, insulin resistance, fertility, carcinogenesis and endothelial function [8]. On the other hand, obesity and insulin resistance stimulates secretion of adipokines and antimicrobial peptides [9,10]. Benachour et al. revealed that cathelicidin mRNA expression was significantly and positively correlated with body mass index and waist circumference. Probably cathelicidin is responsible for the chronic inflammation that is accompanied by the obesity.

It seems that AMP has two faces. On the one hand higher levels of

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cathelicidin may be a marker of inflammation. While on the other side too low levels may be responsible for impaired function of inflammatory system. There is very little data about the role of AMP in diabetes mellitus type 1 (DM1). DM1 is an autoimmune disease, where function of inflammatory system is important in the development and course of the disease [11]. AMP seems to have a beneficial effect on islet function and glucoregulation and may have a positive impact on remission period of the disease [12]. Pound et al. revealed that patients with too low cathelicidin level had no or shorter remission period. On the other side there are data about the relationship of AMP with macroangiopathy in patients with DM1 [13]. In general population cathelicidin correlates with cardiovascular risk factors [9].

Beside improvement in diagnosis and treatment chronic complications still are important clinical problem in this group of patients. Retinopathy is the main reason of blindness, diabetic kidney disease is the leading cause of kidney failure and neuropathy is responsible for increasing amount of amputations [14]. Patients with DM1 still live shorter than their healthy peers [15]. Beside typical risk factors such as hyperglycemia, smoking, dyslipidemia, insulin resistance, the association of inflammatory markers with diabetic complications is also well known [16]. However, the knowledge concerning the association between elevated AMP levels and risk of diabetic microangiopathic complications is still limited.

The aim of this study was to evaluate the relationship between serum cathelicidin levels with the occurance of chronic microangiopathic complications in patients with DM1.

2. Subjects

The study was performed on 62 patients (35 men, 27 women), with type 1 diabetes, recruited consecutively in the Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, with **median** age 30 (interquartile range-IQR 24–38) years, with **median** DM1 duration 12 (IQR 9–17) years, treated with intensive insulin therapy from the onset of the disease. The clinical characteristics of the whole study group are shown in Table 1.

All the subjects were informed about the aim of the study and gave their written consent. The study was approved by local Ethical Committee (Poznan University of Medical Sciences, number 206/16).

Table 1
Clinical characteristics of all patients in the study [data are medians and interquartile range-IQR or n (%)].

Sex M/F	35/27
Age [years]	30 (24–38)
Duration of diabetes [years]	12 (9–17)
Smoking n(%)	18 (29)
Weight [kg]	70 (61–78)
BMI [kg/m^2]	23 (21–25)
Waist circumference [cm]	79 (73–88)
WHR	0.81 (0.76–0.86)
Insulin requirement per kg of body weight [U/kg]	0.63 (0.48–0.72)
HbA1c [%]	8.2 (7.4–8.9)
HbA1c [mmol/mol]	66 (57–74)
TCH[mmol/l]	4.7 (4.1–5.3)
HDL-cholesterol [mmol/l]	1.6 (1.4–2.0)
LDL-cholesterol [mmol/l]	2.4 (2.0–3.0)
TG [mmol/l]	0.98 (0.72–1.24)
Creatinine [μmol/l]	74.2 (65.4–83.1)
GFR (MDRD) [ml/min/1.73 m^2]	99 (90–111)
TSH [mIU/L]	1.6 (1.2–2.3)
hsCRP [mg/l]	0.6 (0.4–1.5)
Cathelicidin [ng/ml]	24.5 (12.5–61.3)
Any microangiopathy n (%)	20 (32)

BMI – body mass index; WHR – waist to hip ratio; TCH – total cholesterol; TG – triglycerides; LDL – low density lipoproteins cholesterol; HDL – high density lipoproteins cholesterol; hsCRP – C-reactive protein; GFR – glomerular filtration rate; HbA1c- glycated hemoglobin; TSH – thyroid stimulating hormone;

2.1. Methods

All the participants completed a standardized questionnaire including details of age, sex, duration of diabetes, current insulin doses, blood glucose self control, medical history, other chronic diseases and medicines, infections during past 6 months, family history regarding diabetes, smoking status, alcohol consumption, diet and physical effort. In order to eliminate stimulated increase of serum cathelicidin patients with chronic or active inflammation (C-reactive protein > 5 mg/l, total leukocytes > 11.0 × 10^9/L), skin infections and additional metabolic disorders (such as liver, kidney failure, hypo- or hyperthyroidism) were excluded from the analysis. To assess presence of microangiopathy patients recruited to the study had duration of DM1 at least 5 years.

All the patients underwent a complete physical examination with anthropometric measurements. Height and weight were measured using the same medical scales for all the patients. Weight was measured to an accuracy of 100 g and height to 0,5 cm. The waist and hip circumferences were assessed using a non-elastic tape to an accuracy of 1 mm. BMI and WHR were calculated from the following equations: BMI = weight [kg] / squared height [m^2] and WHR = waist circumference [cm / hip circumference [cm], respectively.

The insulin requirement per kg of body weight was measured in units of total daily insulin dose [U] (long acting basal insulin and short acting boluses) per body weight [kg].

Blood samples were collected in a fasting state after a period of rest, with minimal occlusion of the vein using the S-Monovette blood collection system. Plasma glucose, serum total cholesterol (TCH), low density lipoproteins cholesterol (LDL), high density lipoproteins cholesterol (HDL), serum triglycerides (TG), thyroid stimulating hormone (TSH) and creatinine levels were measured with the Cobas 6000 biochemistry analyzer (Roche Diagnostics) using enzymatic colorimetric methods. Low density lipoprotein cholesterol (LDL-C) level was calculated by Friedewald formula [17]. HbA1c was assessed by a turbidimetric inhibition immunoassay (Cobas 6000, Roche Diagnostics). GFR was calculated according to the Modification of Diet in Renal Disease Study Equation (MDRD). The C-reactive protein (CRP) concentration was assessed by highly sensitive microparticle enzyme immunoassay. Test sensitivity was 0,03 mg/l.

2.2. Cathelicidin

The serum concentration of cathelicidin was measured with ELISA kit test (ELISA kit is based on the principle of double-antibody sandwich technique to detect Human Cathelicidin) – Shanghai Sunred Biological Technology Company. The measurement of cathelicidin was performed in all patients in the same way – in the morning, in a fasting state after a period of rest. Patients were informed not to smoke before the sampling. The assay sensitivity: 0.286 ng/ml. Assay range: 0.5 ng/ml → 90 ng/ml. Intra-assay Precision: 3 samples with low, middle and high level Human cathelicidin were tested 20 times on one plate, respectively. Inter-assay Precision: 3 samples with low, middle and high level Human cathelicidin were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD / mean × 100. Intra-Assay: CV < 10%. Inter-Assay: CV < 12%.

2.3. Microangiopathy

Screening for diabetic retinopathy was performed using direct ophthalmoscopy through dilated pupils followed, if necessary, by fluorescent angiography. Retinal photographs of each eye were taken using a Fundus Camera VISUSCAM (Zeiss, Germany). After mydriasis with 1% Tropicamide two 45° photographs, macular and nasal, were taken of each eye.

Renal function was assessed by creatinine level, GFR and presence of albuminuria. Assessment of albuminuria was performed by measurement of urinary albumin excretion over 24 h. Albuminuria was