BJMG 11/2 (2008) 11-16
10.2478/v10034-008-0022-0

ORIGINAL ARTICLE

THE TNFα GENE G(–308)A POLYMORPHISM AS A MARKER FOR MYOCARDIAL INFARCTION IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Type-2 diabetes mellitus (DM2) is a major risk factor for the development of coronary artery disease (CAD) and subsequent myocardial infarction (MI). Because the pro-inflammatory cytokine tumor necrosis factor α (TNFα) takes part in several stages of the development of atherosclerosis, we investigated the association of the TNFα gene G(–308)A polymorphism with MI in 142 DM2 patients and in 310 patients who had had DM2 for over 10 years with no clinical signs of CAD and measured the serum TNFα levels in 70 consecutive DM2 patients: 10 with MI and 60 without MI.

We found no association between the G(–308)A polymorphism and MI in DM2 [odds ratio (OR) = 0.8, 95% confidence interval (CI) = 0.5-1.3, $p = 0.4$ for the recessive model and OR = 0.8, 95% CI = 0.2-2.8, $p = 0.7$ for the dominant model]. Significantly higher TNFα serum levels were found in 58 DM2 patients with the GG genotype compared to 12 with the AG genotype (1.13 ± 0.6 ng/L *vs.* 0.53 ± 0.35 ng/L, $p < 0.01$). We concluded that the TNFα gene G(–308)A polymorphism is not a risk factor for MI with DM2 in Slovenes of Slavic origin.

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Key words: Type 2 diabetes mellitus (DM2); Coronary artery disease (CAD); Myocardial infarction (MI); Tumor necrosis factor α (TNFα) gene G(–308)A polymorphism.

INTRODUCTION

Coronary artery disease (CAD) is the leading cause of death in the European Union and in the rest of the developed world [1,2]. Atherosclerosis is generally accepted as a chronic inflammatory condition [3] and growing evidence indicates an important role of chronic inflammation in type 2 diabetes mellitus (DM2) [4]. The pro-inflammatory cytokines may play a central role in the pathogenesis of both CAD and DM2 [3]. An important pro-inflammatory cytokine is tumor necrosis factor α (TNFα) promotes inflammation and signals leading to cell death. It is produced by macrophages, mastocytes, lymphocytes, endothelial cells, and adipocytes [5]. With interleukins (IL) 1 and 6 it has a key role in inflammatory and immune reactions [6]. The TNF plasma concentrations increase in patients affected with premature CAD and is involved in obesity and insulin resistance [7-9], and its expression is regulated at the transcriptional level [10].

The TNFα gene is located on the short arm of chromosome 6 (p21.1-21.3) between the class I and class II regions of the HLA complex. A polymorphism that affects its transcription has been identified in the promoter region of the gene, a guanine-to-adenosine transition at –308 bp in the promoter

region (termed the A allele). In some studies but not in others, an association between this polymorphism and CAD was demonstrated [12-16]. Since this polymorphism has not been tested as a potential marker of MI in DM2, we investigated whether it is associated with increased serum levels of TNFα, and whether it is associated with increased risk for MI in patients.

PATIENTS AND METHODS

We studied Slovene patients of Slavic origin from independent families who had DM2 lasting more than 10 years: 142 with MI (MI group) and 310 DM2 patients with no history of CAD, and no signs of ischemic changes on electrocardiogram and no ischemic changes during submaximal stress testing [17]. The diagnosis of MI was based on the World Health Organization criteria [18]. Patients with MI were included in the study 1-9 months after the acute event. After informed consent was obtained from the patients and control subjects, we conducted a detailed interview. Arterial hypertension and cigarette smoking were defined as binary variables. Patients were classified as having DM2 according to the American Diabetes Association criteria for the diagnosis and classification of diabetes [7]. Body mass index (BMI) was calculated as weight in kilograms divided by the height in square meters. Total serum cholesterol, low density lipoprotein (LDL) cholestetol, high density lipoprotein (HDL) cholesterol and triglycerides serum levels were determined by standard biochemical methods. We analyzed serum TNFα levels in a subpopulation of 70 consecutive DM2 patients: 10 with MI and 60 without MI; in this group there were eight smokers and 62 non smokers, and the mean age was 62.5 ± 10.9 years.

The G(–308)A polymorphism was evaluated by polymerase chain reaction (PCR) using the following primers: sense (5'-AGG CAA TAG GTT TTG AGG GCC AT-3'), antisense (5'-TCC TCC CTG CTC CGA TTC CG-3'). The cycling conditions were: two cycles at 94°C for 3 min., 60°C for 1 min., 72°C for 1 min.; 35 cycles at 94°C for 1 min., 60°C for 1 min., 72°C for 1 min.; two cycles at 94°C for 1 min., 60°C for 1 min. and 72°C for 5 min. The PCR product of 107 bp was digested with the *Nco*I restriction enzyme (Fermentas, St. Leon-Rot, Austria) into two fragments of 87 and 20 bp, respectively. The products of 87 and 20 bp represented the G allele, whereas undigested PCR products represented the A allele. The products were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining [19]. Two of us (KS, DP), who were blinded for the origin of the DNA sample, performed the genotype classification.

Differences in mean values were analyzed by the Student *t*-test. The chi-square test was used to compare discrete variables and genotype distributions. Genotypic odds ratios (ORs) for MI with 95% confidence intervals (CIs) with two-tailed *p* values were calculated by the chi-square test. Statistical analysis was performed using the SPSS program 15 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The characteristics of the patients studied are summarized in Table 1. The patients with MI were younger, predominantly male and had a higher incidence of cigarette smoking than those without MI. They also had higher total cholesterol and LDL cholesterol levels, and longer duration of DM2 (Table 1). There were no significant differences in the incidence of hypertension, HDL cholesterol and triglyceride levels between the two groups (Table 1).

The frequency of the G(–308)A polymorphism is shown in Table 2. These were compatible with the Hardy-Weinberg expectations. There was no association between the polymorphism and MI in DM2 patients (OR = 0.8, 95% CI = 0.5-1.3, p = 0.4 for the recessive model and OR = 0.8, 95% CI = 0.2-2.8, p = 0.7 for the dominant model). The allele frequencies in patients with MI were: G 247 (87.0%) and A 37 (13.0%), and allele frequencies in patients without MI were: G 550 (88.7%) and A 70 (11.3%); the difference was not statistically significant.

We analyzed serum TNFα levels in 70 consecutive DM2 patients. Significantly higher levels were found in 58 patients with the GG genotype compared to 12 patients with the AG genotype (1.13 ± 0.6 ng/L *vs.* 0.53 ± 0.35 ng/L:, p <0.01). We found no statistically significant differences between 10 DM2 patients with MI and 60 DM2 patients without MI [0.61 ± 0.528 ng/L *vs.* 1.10 ± 1.23 ng/L, p = no significance (n.s.)]. Moreover, we failed to demonstrate statistically

Table 1. Characteristics of patients with type 2 diabetes mellitus

Parameters	DM2 With MI	%	DM2 Without MI	%	*p* Value
Number of patients	142		310		
Age [in years ± standard deviation (SD)]	61.0 ± 11.9		66.2 ± 9.8		<0.001
Males	95	66.9	144	46.4	<0.001
Females	47	33.1	166	53.6	<0.001
Body mass index (kg/m^2 ± SD)	28.7 ± 3.9		29.0 ± 4.6		0.4
Arterial hypertension	100	70.4	219	70.6	0.8
Normotensive DM2 patients	42	29.6	91	29.4	0.9
Systolic BP (mm Hg ± SD)	147 ± 21		143 ± 22		0.1
Diastolic BP (mm Hg ± SD)	83 ± 10		84 ± 10		0.5
Smoking habit	58	40.8	37	11.9	<0.001
DM2 duration (years ± SD)	21.7 ±7.7		17.9 ± 8.0		0.001
Total serum cholesterol (mmol/L ± SD)	5.7 ± 1.6		5.3 ± 1.3		0.01
HDL serum cholesterol (mmol/L ± SD)	1.1 ± 0.3		1.2 ± 0.4		0.12
LDL serum cholesterol (mmol/L ± SD)	3.6 ± 1.5		3.2 ± 1.0		0.001
Serum triglyceride (mmol/L ± SD)	2.3 ± 1.3		2.4 ± 1.6		0.8

Table 2. Genotype and allele distribution of the TNFα *gene* polymorphism in type 2 diabetes mellitus patients with myocardial infarction and patients without myocardial infarction

TNFα Genotype	DM2 With MI	%	DM2 Without MI	%	OR	95% CI	*p* Value
Genotype GG	109	76.8	247	79.7	0.8	0.5-1.3[a]	0.4[a]
Genotype GA	29	20.4	56	18.1	0.8	0.2-2.8[b]	0.7[b]
Genotype AA	4	2.8	7	2.2			
Total	142	100.0	310	100.0			
G allele	247	87.0	550	88.7			0.5
A allele	37	13.0	70	11.3			
Total	284	100.0	620	100.0			

[a] *p* Value and OR for recessive model (TNFα: AA *vs.* GA plus GG).
[b] *p* Value and OR for dominant model (TNFα: AA plus GA *vs.* GG).

significant differences in serum TNFα levels between eight smokers and 62 non smokers (1.35 ± 0.8 ng/L *vs.* 1.11 ± 1.22 ng/L, p = n.s.). Similarly, no statistically significant correlation between age and serum TNFα levels were demonstrated ($p = 0.9$, correlation coefficient = 0.004).

DISCUSSION

We found no association between the TNFα gene G(–308)A polymorphism and MI in our Slovene patients with DM2. This may be the first report of this negative association. The G(-308)A polymorphism has been related to MI [13], to unstable angina [16], and ST-elevation MI (STEMI) [20]. Other studies failed to demonstrate an association between this polymorphism and MI [12,14,15]. This discordance may be due to the multifactorial nature of the MI, to differences in study design or to genetic heterogeneity within and between the populations studied. Moreover, we found significantly higher serum TNFα levels in DM2 patients with the GG genotype than with AG genotype. Several environmental and genetical factors (*i.e.,* age, cigarette smoking, ischemia) influence serum TNFα levels [9,21,22]. The G(–308)A polymorphism is most probably a functional polymorphism, since it has been reported to affect the serum TNFα levels [21,22]. The A allele was associated with decreased TNFα concentrations in offspring less than 18 years of age and males who were not overweight [BMI <25 kg/m (2)]. Moreover, cigarette smoking was reported to lower TNF concentrations, but in our study we did not find an effect of smoking on TNF concentration. It has been speculated that cigarette smoking abolishes TNFα cellular release from cells [21,22].

We studied only DM2 patients who had experienced a MI 1-9 months earlier, and our results may be affected by survival bias. Moreover, the exclusion of CAD on the basis of a negative history of MI or angina pectoris, and absence of ischemic changes on electrocardiograms and during exercise stress testing, was disadvantaged by the possibility that some of the patients without MI may have experienced asymptomatic CAD. In a recent study, 18% of asymptomatic DM2 patients with a negative result of exercise stress testing, presented silent CAD with significant (≥70%) angiographically-documented coronary stenosis [17]. In conclusion, the G(–308)A polymorphism of the TNFα gene is not a risk factor for MI with DM2 in Slovenes of Slavic origin.

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