Clinical Biochemistry 47 (2014) 574–577

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

GSTM1-null and GSTT1-null genotypes are associated with essential arterial hypertension in patients with type 2 diabetes

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ARTICLE INFO

Article history:
Received 19 January 2014
Received in revised form 3 March 2014
Accepted 6 March 2014
Available online 28 March 2014

Keywords:
Oxidative stress
Genes
Gene polymorphism
Glutathione S-transferases
GSTM1
GSTT1
GSTP1
MnSOD
Essential arterial hypertension
Diabetes mellitus type 2

ABSTRACT

Objective: To evaluate whether the genetic polymorphisms of glutathione S-transferases M1 (GSTM1) and T1 (GSTT1), Ile105Val of the GSTP1 (rs947894), and the Val16Ala polymorphism of the MnSOD (rs4880) are associated with essential arterial hypertension (EAH) in Caucasians with type 2 diabetes.

Design and methods: 1015 Slovenian subjects (Caucasians) with type 2 diabetes with/without EAH were enrolled in the cross-sectional study. Genotypes were determined by multiplex PCR amplification and PCR-restriction fragment length polymorphism method.

Results: In the cross-sectional study, GSTM1-null genotype and GSTT1-null genotype were associated with EAH in subjects with type 2 diabetes (59.0% vs. 50.3%, $p = 0.007$; 28.5% vs. 20.7%, $p = 0.008$; consequently).

Conclusion: After adjustment for age, body mass index, and hsCRP level, GSTM1-null and GSTT1-null genotypes were found to be independent risk factors for the development of EAH in Slovenian patients with type 2 diabetes.

Introduction

In most populations, arterial hypertension affects 25% of adults and is a major risk factor for death from cardiovascular disorders [1]. The most prevalent form of hypertension is essential arterial hypertension (EAH). EAH is a complex disorder influenced by various genetic and environmental factors [2]. Several complex physiological systems have been so far implicated in the development of EAH, including increased oxidative stress [2].

Despite the fact that data from animal studies support a causative role for oxidative stress in the pathogenesis of EAH, there is still no solid evidence that oxidative stress causes EAH in humans [3–7].

Increased oxidative stress markers have recently been reported in hypertensive subjects in comparison with normotensive subjects [8]. The increased oxidative stress in subjects with EAH is a consequence of the increased production of reactive oxygen species. The oxidative stress in EAH was reported to be increased not only due to increased ROS production but also due to decreased antioxidant defense mechanisms [9–11]. Therefore, it could be assumed that the variability of the oxidative stress genes could increase the risk of EAH in type 2 diabetes.

Endogenous antioxidant enzymes exist to inactivate reactive oxygen species. Glutathione S-transferases are phase II detoxifying enzymes that catalyze the conjugation of reduced glutathione to a wide range of electrophilic substrates and represent a major protective mechanism against oxidative stress [12]. Human glutathione S-transferases include members of eight classes, and the most commonly studied are the deletions of the GSTM1 and GSTT1 genes, and the GSTP1 Ile105Val polymorphism [13]. The GSTM1-null and GSTT1-null variants result in the absence of the functional enzyme and consequently greater vulnerability to oxidative stress [13].

Manganese mitochondrial superoxide dismutase (MnSOD) protects the human body from oxidative stress by converting the toxic superoxide anion ($O2^-$) into less toxic hydrogen peroxide (H2O2) [14]. In extracellular-SOD knockout mice, blood pressure was reported to be significantly higher than that seen in wild-type mice [6,7]. The Val16Ala polymorphism of the MnSOD (rs4880) is predicted to alter the secondary structure of MnSOD [15]. In investigations in vitro the alanine MnSOD mitochondrial targeting sequence variant was associated with 30% to 40% higher MnSOD activity in the mitochondrial matrix

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http://dx.doi.org/10.1016/j.clinbiochem.2014.03.012
0009-9120/

compared to the valine MnSOD variant [16]. The rs4880 polymorphism of MnSOD has recently been reported to be associated with several diseases [13,17].

Antioxidant enzymes represent an important protective mechanism against the damage caused by oxidative stress. Due to the fact that genetic variability of the glutathione S-transferases and MnSOD was implicated in the pathogenesis of chronic complications of diabetes, it is hypothesized that the variability of the oxidative stress genes could increase the risk of EAH in type 2 diabetes patients [18]. Moreover, involvement of genetic polymorphisms of the oxidative stress genes in EAH has already been reported, including GST genes, with contrasting results [9,10,19–23].

The aim of the present study was to determine the possible association between polymorphic deletions of the GSTT1, GSTM1, the rs947894 of the GSTP1, and the Val16Ala polymorphism of the MnSOD (rs4880) and EAH in Slovenian patients (Caucasians) with type 2 diabetes.

Patients and methods

Patients

In this cross-sectional case–control study, consecutive patients with type 2 diabetes from the outpatient clinics for patients with diabetes from central and north-eastern regions of Slovenia were enrolled. The study included 1015 subjects with type 2 diabetes. The research protocol was approved by The National Medical Ethics Committee. Patients were classified as having type 2 diabetes according to the current American Diabetes Association criteria [24]. After the informed consent was obtained from the patient, a detailed interview was made concerning smoking habits, the duration and treatment of diabetes, arterial hypertension, and hyperlipidemia. Patients were asked whether they were smokers at the time of recruitment ("current smoker"). Moreover, the body mass index (BMI), defined as the individual's body mass divided by the square of their height, was calculated as well.

The cases (subjects with type 2 diabetes with EAH) and control subjects (subjects with type 2 diabetes without EAH) came from independent families. However, genetic testing was not performed to exclude familial connection. All the subjects had home and ambulatory blood pressures measured: systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the right upper arm of the patients were measured while they were sitting (2 consecutive measurements). Subjects with type 2 diabetes with systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 80 mm Hg and/or subjects who were using antihypertensive drugs were considered to be hypertensive [25]. Secondary causes of arterial hypertension were excluded according to normal clinical exam (no systolic murmur above renal arteries) and normal serum electrolytes (exclusion of renal failure and hyperaldosteronism) [25].

Genotyping

Genomic DNA was extracted from 100 μl of whole blood using a Qiagen isolation kit. Genotypes of GSTM1 and GSTT1 were determined by multiplex PCR amplification, as described previously [20]. For GSTT1-0/GSTM1-0 genotype no bands were obtained, necessitating the use of albumin as internal positive control, in order to distinguish the null genotype from aborted PCR reactions. Amplification results in a 480 bp GSTT1 fragment, 215 bp GSTM1 fragment and 350 bp albumin fragment [26]. By using this protocol it was not possible to distinguish homozygous and heterozygous carriers of GSTM and GSTT alleles.

The GSTP1 genotype was evaluated by PCR using the following primers: forward 5′-ACC CCA GGG CTC TAT GGG AA-3′ and reverse 5′-TGA GGG CAC AAG AAG CCC CT-3′ as described previously [27]. The PCR product was then digested with 3U Alw261 (New England Biolabs, Hertfordshire, UK). In homozygotes for A allele a fragment of 176 bp is seen. Restriction site appears when the G allele is present and in GG homozygotes two fragments are seen (91 bp + 85 bp). In heterozygous subjects three fragments are seen (176 bp, 91 bp, 85 bp).

Biochemical analyses

Glucose, high sensitivity C-reactive protein (hsCRP), total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), and triglycerides were determined by standard biochemical methods.

To assess oxidative stress in a randomly selected subjects with type 2 diabetes with arterial hypertension (86 subjects) and without arterial hypertension and (84 subjects), serum levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) were measured. Serum levels of 8-OHdG were measured with the "Highly sensitive 8-OHdG check" enzyme-linked immunosorbent assay (ELISA) kit (IBL International GMBH, Hamburg Germany).

Statistical analysis

Data are expressed as means ± standard deviations or frequencies (percentages). The chi-square test was used to compare discrete variables. Continuous clinical data were compared by an unpaired Student's t test (normal distribution by Kolmogorov–Smirnov test) or by Mann–Whitney U test (for variables without normal distribution by Kolmogorov–Smirnov test).

Multiple logistic regression analysis was performed for the evaluation of the independent effect of genotypes on arterial hypertension. A $p < 0.05$ was considered statistically significant. A statistical analysis was performed using the SPSS program for Windows version 20 (SPSS Inc. Illinois).

Results

The characteristics of subjects with type 2 diabetes with regard to the presence/absence of EAH are listed in Table 1. Subjects with EAH were older and they had higher body mass index (BMI) in comparison with subjects without EAH (Table 1). Higher hsCRP levels were observed in subjects with EAH in comparison with subjects without EAH (Table 1).

Table 1
Characteristics of patients with type 2 diabetes with EAH (cases) and without EAH (controls).

Characteristics	Cases	Controls	p
Number	629	386	–
Age (years)	64.1 ± 9.4	61.5 ± 11.9	<0.001
Male sex (%)	325 (51.7)	196 (50.8)	0.7
Duration of diabetes (years)	14.9 ± 8.9	15.2 ± 10.2	0.7
Age of onset of diabetes (years)	51.1 ± 10.9	50.4 ± 10.9	0.3
Systolic blood pressure (mm Hg)	149.6 ± 20.5	128.3 ± 21.5	<0.001
Diastolic blood pressure (mm Hg)	85.9 ± 11.8	78.9 ± 7.3	<0.001
BMI (kg/m^2)[a]	29.4 (26.7–33.0)	27.5 (25.1–30.1)	<0.001
Waist circumference	110 (102–118)	102 (95.5–108)	<0.001
Smokers (%)	62 (9.9)	35 (9.1)	0.8
Glucose (mmol/l)	8.37 ± 2.43	8.42 ± 2.87	0.87
Hba1c[b]	7.99 ± 1.05	7.84 ± 1.18	0.7
hsCRP (mg/l)[c]	2.4 (1.2–3.5)	1.55 (0.8–3.8)	0.003
8-OHdG (ng/ml)[d]	1.56 (1.30–1.74)	1.50 (1.30–1.70)	0.5
Antihypertensive drugs	558 (88.7)	0	<0.001
Hypolipemic drugs	415 (65.9)	242 (62.7)	0.07
Total cholesterol (mmol/l)	4.7 ± 1.1	4.8 ± 1.1	0.8
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.2 ± 0.4	0.5
LDL cholesterol (mmol/l)	2.6 ± 0.9	2.5 ± 0.9	0.3
Triglycerides (mmol/l)	2.2 (1.3–3.3)	2.1 (1.0–2.9)	0.6

Continuous variables were expressed as means ± standard deviations when normally distributed and as median (interquartile range) when asymmetrically distributed.
[a] BMI — body mass index.
[b] Hba1c — glycated hemoglobin A1c.
[c] hsCRP — high sensitive C reactive protein.
[d] 8-OHdG — 8-hydroxy-2-deoxyguanosine.